DOCTORAL (PhD) DISSERTATION

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DIETARY INFLUENCE OF FIBER ON THE ENERGY AND AMINO ACID DIGESTIBILITY AND ITS CONSEQUENCES FOR DIET FORMULATION IN GROWING PIG

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2. Introduction
The use of by-products in swine nutrition will always remain important to reduce feed costs in swine production. A monogastric livestock like the hog will remain forever competitors with humans for food if cereal production is limited. However, cereal by-products like wheat bran and others like soylhulls, sunflower meal, rapeseed meal or distillers grain from ethanol production will be increasingly available for the feed industry. New elements in crop production in recent years are now utilized for energy like bioethanol, biodiesel and biogas. The limited availability and increasing cost of energy has started to compete with food and feed ingredients.

In the future the feed industry will have to compete not only for grain but for the by-products as well. If the feed industry is not ready to use these by-products they will simply be burned to gain energy directly or be used for biogas production for further energy yield.

Some of the by-products are available in wet form. In this case the transportation cost also can be a limiting factor to use it for animal feeding. If the by-product available is in dry form it can easily be used by the feed industry. The process by which by-products are produced concentrates the fiber fraction. To use these ingredients the feed industry has to evaluate them precisely - first of all not to lose animal performance and secondly to evaluate precisely the value of the by-products in the least cost formulation. The findings of recent research into the digestion of fiber fractions should be incorporated into swine formulation concepts.

The role of dietary fiber in pig nutrition has been investigated for some decades. The first concept on dietary fiber developed the interest in ‘unavailable’ carbohydrates in the late 1920s by McCance and Lawrence (1929). The term of fiber denotes a number of chemically different materials, which are not able to be digested by the endogenous enzymes of livestock. Originally, the dietary fiber concept focused on the plant cell wall
as the main source of undigestible material, but recently resistant starch and oligosaccharides have emerged as other important sources of fermentation substrates for the large intestinal micro flora (Asp, 1996). There is evidence now that dietary fiber has some undesirable and also a number of beneficial effects on the digestive physiology of monogastric animals. Fiber rich by-product can help to maintain better gut health.

Increasing demand for cereals in human consumption forces the animal nutritionist to use more by-products in diets of monogastric animals (Verstegen and Tamminga, 2005). In recent years the use of fibrous by-products has significantly increased in swine diets resulting in increased dietary fiber content. Pigs are capable of converting these by-products or ‘wastes’ of all sources (which will normally be discarded by humans) into wholesome animal protein useful to the human being (Adesenhinwa, 2008).

Priority and increased demand of high energy cereals for direct human use and increased availability of fiber rich ingredients from by-products (e.g. wheat bran, soyhulls, sugar beet pulp, corn gluten) have promoted an increased utilization of fiber rich feedstuffs in pig nutrition (Noblet and Le Goff, 2001). The other reason is to maintain a competitive hog feed price and production cost by utilizing these by-product. Another reason for increased interest in the role of dietary fiber in pig nutrition is due to the development of new analytical and experimental methods. In the past the main interest was focused on the effect of crude fiber on health and digestion, it is now possible to estimate more accurately the consequences of specific dietary fiber properties on the digestive process in the different sections of the gastrointestinal tract (GIT) (Souffrant, 2001). New analytical methods were developed to better describe the fiber fraction like the van Soest neutral detergent fiber (NDF) the acid detergent fiber (ADF) acid detergent lignin (ADL), soluble and insoluble fiber determination, non
starch polysaccharide (NSP), total dietary fiber (TDF). There has been a rapid growth in the development of robust and reproducible enzymatic-chemical methods for the determination of fiber components over the last two decades (Bach-Knudsen, 1997). Furthermore different experimental (surgery) techniques have become available to detect the digestion process in different segments of the GIT (Laplace et al., 1985; van Leeuwen et al., 1988; Köhler, 1992, Köhler et al., 1992). These techniques can help to understand the mechanisms governing nutrient digestibility and moreover the interactions between nutrients in the absorption processes.

Several studies report that dietary fiber depresses the digestible protein and energy supply. Moreover, some results have been published on the effect of fiber interaction, particularly with dietary fat, on the nutrient digestibility (Shi and Noblet, 1994; Bakker, 1996). In this respect the presence of fiber did not only interfere with the digestibility of other nutrients, but probably altered the effect of other nutrients on digestion as well (Bakker, 1996). In theory, performance of growing and finishing pigs fed dietary fiber will not decline if one formulates such that pigs consume adequate amounts of net energy (NE), ileal digestible amino acids and other essential nutrients (Just, 1984). However, it has also been demonstrated that pigs performed worse when offered diets with a similar calculated net energy supply but composed of by-products plus supplementary fat, compared to pigs given diets based on cereals or by-products without supplemented fat (Jongbloed et al., 1986; Bakker, 1996). It was suggested that either the energy contribution of the fat was overestimated or the fat and fiber interaction in energy (DE, ME) supply was underestimated. Thus, in case of any fiber interaction, nutrient requirements of growing and fattening pigs must be changed when high fiber diet is fed. For that reason, quantitative data on the effect of dietary fiber on digestibility and interaction
of fiber with other nutrients are crucial in order to re-evaluate the present feed evaluation systems for their accuracy when large amounts of fiber are present in the pig diet. In diet formulation, knowledge on the effect of inclusion of different by-products, including interactions of nutrients, has to be integrated.

For environmental protection and economic reasons something needs to be done with the agro-industrial by-products. Primarily they can be used for human consumption but certain parts will be available for either the feed industry or energy sector. It is concluded that fiber rich by-products have been used more and more extensively in pig production in the past years due to economical reasons to better utilize the agro-industrial wastage. This increased usage is essential for animal protein production and has brought competitive advantage to the pork industry while increasing the challenge to evaluate these ingredients more precisely.
3. Literature review
3.1 Definition and determination of fiber

Initially dietary fiber was defined as the skeletal remains of plant cells in the diet, which are resistant to hydrolysis by the digestive enzymes of man (Trowell et al, 1976). The Weende crude fiber (Henneberg and Stohmann, 1859), the van Soest fiber (van Soest, 1973) and total dietary fiber (TDF) are the three predominant methods of fiber characterization that can be applied to animal diets (Johnston et al., 2003). The Weende and van Soest methods are still the methods frequently used to describe the indigestible carbohydrate fraction in swine diets (Bach-Knudsen, 1997). The „crude fiber” method (Hennberg és Stohmann, 1859) is able to determine partially the cellulose lignin cutin and suberin content followed by an acetic and alkaline hydrolysis. The residuum of the Weendei fiber analysis depends on feed ingredients contains variable amounts of cellulose 30 - 100 %, pentosans 14 - 20 %, lignin 16 - 90 % (Gidene and Lebas 2002). Van Soest method is used widely alongside Weendei analysis. The limitation of the NDF method is that part of the measured fraction also contain crude protein (1 - 20 % in grains) as well as some starch and pectin. Pectin and other water soluble NSPs are lost during NDF determination, enzymatic preparation not fully standardized, and some hemicellulose could remain in ADF fraction (Dégen et al. 2007). In the van Soest method hemicellulose, cellulose and lignin (NDF, ADF, lignin) are measured, while the crude fiber method gives a measure for cell wall content somewhere between the neutral detergent fiber and acid detergent fiber (Figure 1).
Figure 1. Schematic representation of the carbohydrate fraction of the diet
However, none of these methods analyze the pectin content and do not yield information about the hemicellulose composition, such as neutral sugars. These shortfalls are eliminated by the methods of analysis of total dietary fiber, which is a more appropriate measure of fiber because it accounts for water-soluble fiber (SDF) such as pectins, β-glucans, fructans and other soluble sugars and insoluble fibers (IDF). TDF is used particularly in human nutrition, but it has been used in some pig nutrition studies as well in the recent past. Soluble and insoluble fibers represent the polysaccharides which are determined by fractional extraction controlled by changing the pH of the solution (FAO, 1998). The separation of soluble and insoluble fractions proved very useful in understanding the physiological properties of dietary fiber; however, it is not a distinct chemical separation and depends on the condition of extraction (Asp et al., 1992). The fraction, called non-starch polysaccharides (NSPs), is the total dietary fiber fraction of feedstuffs excluding lignin (Figure 1). According to de Lange (2000), over 100 monosaccharides are found in nature but only about nine of these are the predominant building blocks of NSP. However, the physiological properties of dietary fiber are poorly predictable from the monomeric composition of the dietary fiber constituents (de Lange, 2000; Souffrant, 2001). It appears that more conventional measures of NSPs, such as neutral detergent fiber or soluble and insoluble dietary fiber, provide for reasonable means to predict the effect of NSPs on nutrient and energy digestibility in various types of pig feed ingredients (Noblet and Henry, 1993; Bakker, 1996; Bach-Knudsen, 1997). Crude fiber means only the analytical end results of Weende analysis and means nothing in terms of precise chemical components. The constituents of the fiber components vary from ingredient to ingredient. A more precise description is necessary to describe fiber fraction when complete feed needs to be formulated or evaluated. In the coming sections
the impact of fiber will be discussed in terms of traditional versus recent concepts of dietary fiber.

3.2 Effects of dietary fiber

Traditional fiber concept declared that the presence of fiber in the diet decreases the incidence of constipation by stimulating the peristaltic movement of the gut and the speed of emptying the gut content (Fekete, 1995). The contribution of digestible fiber to the nutrient supply of the pig is far less than dietary protein or fat; however it has a significant physiological impact on the entire gut function and thus on the utilization of other nutrients (Schmidt, 1995; Fekete, 1995). Due to the physiological and antinutritional role of fiber there is an optimum fiber supply in terms of feed conversion depending on the age of the pig (Fekete, 1995).

On the other hand as reviewed by de Lange (2000) non-starch polysaccharides may affect the production and activity of digestive enzymes, intestinal morphology (including cell proliferation), the microbial population in various segments of the gut, and secretion of certain hormones (including insulin, glucagon, gastric inhibitory polypeptide and possibly secretin and cholecystokinin). It is difficult to describe with certainty the effects of various fiber components (SDF or IDF for instance) on digestibility, because they are not homogenous (Johnston et al., 2003). However, it was reported that soluble dietary fibers increase the viscosity of the digesta (Mosenthin et al., 2001; Noblet and Le Goff, 2001). Increased viscosity in the small intestine might slow gut transit time due to suppressed intestinal contractions (Cherbut et al., 1990) which in turn leads to less mixing of dietary components with endogenous digestive enzymes (Johnston et al., 2003). Le Goff et al. (2002) reported that in growing pigs and sows the mean retention
time in the gastro-intestinal tract of pigs was shorter for diets containing maize bran or wheat bran (200 g DF/kg DM) compared to a control diet (based on corn-soybean meal, 100 g DF/kg DM) and to a diet containing sugar beet pulp (200 g DF/kg DM), which provides pectin and inulin. As a conclusion, unlike soluble fibers, insoluble fibers shorten the transit time of the digesta through the gastrointestinal tract. It is often suggested, that this short transit time allows the digestive enzymes less time for degradation. Also less time available for fermentation in the hindgut and the rapid passage of digesta may diminish the effectiveness of this process (Morel at al., 2006).

Wilfart et al. (2007) used diets with low, medium and high fiber content by replacing wheat and barley with wheat bran and found that dietary treatments had no influence on the apparent ileal digestibility of nutrients; however, increasing the insoluble fiber content of the diet negatively affected the fecal digestibility of CP, ether extract and energy (Wilfart et al. 2007)

In case of high insoluble dietary fiber, the passage time is only reduced in the hind gut, and the time it takes for the digesta to pass through the small intestine is either not affected or even prolonged. Generally the amount of digesta is certainly larger in case of diets with high insoluble fiber content and the total transit time is shorter with increasing amounts of digesta that flows into the caecum (Kesting et al., 1991). On the other hand it has been shown that the digestibility of dietary energy and nutrients, especially the dietary fiber fraction, increased with the increase in body weight from growing to finishing pigs and was even higher in adult sows (Noblet et al., 1994; Noblet and Bach-Knudsen, 1997; Le Goff et al., 2002).

In conclusion the changes in the physical characteristics of the intestinal contents due to presence of specific fiber components may influence gastric emptying, dilute gastrointestinal enzymes and absorbable compounds in the
gut and slow the diffusion or mobility of enzymes, substrates and nutrients to the absorptive surface (FAO, 1998). However, there is some evidence, that the effects of purified nutrients are different when compared to nutrients as constituents in feed. In the case of two or more nutrients the effect of each one can be modified, therefore the interaction of nutrients can also affect the digestion. Aside from factors resulting in reduction of digestibility, there are several beneficial effects of dietary fibers on, for instance, the microflora of the gastro-intestinal tract and thus the gut health, ammonia emission from slurry and animal welfare. Structure and function of the gut is directly influenced by fiber in the diet.

Some of the specific components of dietary fiber may have significant impact on animal health. Oligosaccharides such as mannan-oligosaccharides, fructo-oligosaccharides, galacto-oligosaccharides, are non-digested and can limit the population of pathogenic bacteria by enhancing the beneficial microbes in the gut (Hathaway, 2000; Pettigrew, 2000). Proliferation of epithelial cells is supported by butyrate, which is produced in the fermentation of dietary fiber and absorbed in the gut (Mosenthin et al., 2001; Montagne et al., 2003). Feeding dietary fiber supports hindgut fermentation and reduces the potential for non-pathogenic diarrhea to occur (Johnston et al. 2003). Fermentable fiber reduces the severity of Salmonella Typhimurium infection (Correa- Matos et al., 2003). Whitney at al. (2006) reported reduced severity of intestinal infections of Lawsonia Intracellularis by diets containing DDGS, which are high in hemicellulose. It seems that piglet diets rich in insoluble fiber protect more efficiently against pathogenic bacteria than diets high in soluble ones (Hederman et al., 2006). However it should be noted, that to discuss all possible mode of action of dietary fiber on gut health is out of the scope of the thesis.
Dietary fiber can affect the digestive conditions in the stomach and small intestine even before it reaches the large intestine (Drochner et al., 1984). Thus, fiber in the diet of growing and fattening pigs may reduce nutrient digestibility due to reduced nutrient absorption and (or) increased endogenous excretion (Lenis et al., 1996). Accordingly, when feeding high fiber diets the digestibility of protein can be different if it is characterized by true vs. apparent or by ileal vs. total tract digestibility. Therefore the discussion on the effect of dietary fiber on the digestibility of protein is also arranged in two sections.

3.3 Effect of dietary fiber on total tract and ileal protein digestibility

Protein is one of the most biologically and economically important nutrients and therefore it is key to supply the protein and/or amino acid requirements as accurately as possible. It has been demonstrated that the nutritional importance of protein and amino acids absorbed in the large intestine is minor, however almost all of this N is rapidly excreted via urine, mainly in the form of urea (Zebrowska, 1975). Consequently ileal digestible protein and/or amino acids provide the real requirement of a pig. However, the difference between total tract (fecal) and ileal digestibility shows that fermentation occurs in the large intestine. Considering that dietary fiber enhances the fermentation processes and supplies energy as short chain fatty acids, both ileal and total tract digestibility would be interesting to study.

*Soluble and insoluble fiber*

Literature data on the effect of different dietary fibers on digestibility of protein is presented in Table 1. In the case of inclusion of rapidly fermentable NSPs such as from sugar beet pulp or pectin in the pig diet, both
fecal and ileal apparent digestibility of protein and/or amino acids decreased (Dierick et al., 1983; Mosenthin et al., 1994; Zervas and Zijlstra, 2002a). The reduction in ileal digestible protein supply due to soluble fiber might be caused by pectin and other gel-forming polysaccharides by reducing absorbed by amino acids and peptides, withholding these from absorption (Mosenthin et al., 1994). However, in a study performed by den Hartog et al. (1988) no effect of 50 g/kg pectin was found on either ileal or fecal apparent digestibility of dietary protein and amino acids. The lack of response may have been caused by feeding a basal diet composed of natural NSP-containing ingredients (corn, barley, soybean meal), whereas highly digestible semi-purified cornstarch-based diets, which are much more sensitive to the additional supply of pectin, were used in the studies by Dierick et al. (1983) and Mosenthin et al. (1994).

Dietary inclusion of NDF is also believed to negatively affect both the ileal and total tract apparent digestibility of protein and amino acids (Sauer et al., 1991; Schultze et al., 1994; Lenis et al., 1996; Yin et al., 2000; Table 1). Although some studies report a threshold effect of insoluble fiber at approximately 100 g/kg inclusion (Li et al., 1994), Dilger et al. (2004) found a linear reduction in both apparent and true digestibility of amino acids, and in particular of lysine by NDF inclusion from soyhulls (in a range of 27 to 76 g/kg of diet). However, it has to be admitted that although in soyhulls approximately 45 % of dry matter is cellulosic in nature (Lo, 1989), it is usually categorized as highly fermentable carbohydrate (Bakker, 1996).
Table 1. Some literary data on the effect of different type of fibers on digestibility of crude protein and amino acids

<table>
<thead>
<tr>
<th>Authors</th>
<th>Fiber source</th>
<th>Fiber type</th>
<th>BW (kg)</th>
<th>protein digestibility</th>
<th>amino acids digestibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mosenthin et al., 1994</td>
<td>citrus pectin</td>
<td>pectin 75 g/kg</td>
<td>70</td>
<td>ID ↓</td>
<td>FD and ID ↓ for all AAs*</td>
</tr>
<tr>
<td>Den Hartog et al., 1988</td>
<td>pectin 50 g/kg</td>
<td>cellulose 50 g/kg</td>
<td>40</td>
<td>FD and ID Ø</td>
<td>FD and ID Ø for all AAs*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>straw meal 50 g/kg</td>
<td>40</td>
<td>FD Ø and ID ↓</td>
<td>FD ↓ for Cys, Gly; ID ↓ for Iso, Lys, Phe, Thr, Val, Ala, Asp, Glu, Tyr, FD and ID Ø for rest of AAs</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>40</td>
<td>FD and ID Ø</td>
<td>FD and ID Ø for all AAs*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schultz et al., 1994</td>
<td>purified NDF from wheat bran</td>
<td>NDF 0, 60, 120 180 g/kg</td>
<td>25</td>
<td>ID ↓</td>
<td></td>
</tr>
<tr>
<td>Lenis et al., 1996</td>
<td>purified NDF from wheat bran</td>
<td>NDF 18 vs. 80 5 g/kg</td>
<td>27</td>
<td>FD and ID ↓</td>
<td>ID ↓ for all AAs*</td>
</tr>
<tr>
<td>Dilger et al., 2004</td>
<td>soyhulls</td>
<td>NDF 27-76 g/kg</td>
<td>35</td>
<td>FD and ID Ø</td>
<td>ID** ↓ for Arg, His, Iso, Lys, Phe, Asp, Ser, Tyr, ID** Ø for rest of AAs</td>
</tr>
<tr>
<td>Zervas and Zijlstra, 2002a</td>
<td>soyhull vs. sugarbeet pulp</td>
<td></td>
<td>31</td>
<td>FD Ø</td>
<td></td>
</tr>
<tr>
<td>Bach Knudsen and Hansen, 1991</td>
<td>wheat by-products</td>
<td>NSP 30-54 g/kg, lignin 4-8 g/kg</td>
<td>40-50</td>
<td>FD ↓ and ID Ø or ↓</td>
<td>FD ↓ for all AAs* ; ID ↓ for Leu, Gly, ID Ø for rest of AAs</td>
</tr>
<tr>
<td></td>
<td>oat by-products</td>
<td>NSP 34-96 g/kg, lignin 9-13 g/kg</td>
<td>40-50</td>
<td>FD and ID ↓</td>
<td>FD ↓ for all AAs* ; ID ↓ for Leu, Gly, ID Ø for rest of AAs</td>
</tr>
<tr>
<td>Sauer et al., 1991</td>
<td>10 % cellulose</td>
<td></td>
<td>50</td>
<td>FD and ID ↓</td>
<td>FD ↓ for all AAs*, ID ↓ for Leu, Gly, ID Ø for rest of AAs</td>
</tr>
<tr>
<td></td>
<td>10 % barley straw</td>
<td></td>
<td>50</td>
<td>FD and ID ↓</td>
<td>FD ↓ for all AAs*, ID ↓ for Leu, Gly, ID Ø for rest of AAs</td>
</tr>
<tr>
<td>Yin et al., 2000</td>
<td>wheat by-products</td>
<td>insoluble NSP 75-184 g/kg</td>
<td>26</td>
<td>FD and ID ↓</td>
<td>ID ↓ for all AAs* except for Arg</td>
</tr>
</tbody>
</table>

BW – body weight; FD - faecal digestibility, ID - ileal digestibility (both refer to apparent digestibility), ↓ - reducing effect, Ø - no effect
* Arg, His, Iso, Leu, Lys, Met, Phe, Thr, Try, Val, Ala, Asp, Cys, Glu, Gly, Pro, Ser, Tyr
** Both apparent and true ileal digestibility
Therefore, the effect of pure cellulose and a ‘mixed’ fiber from natural sources may not be comparable. The impact of purified dietary fibers on gut function are not necessarily similar to those of intact fibers in feedstuffs, presumably due, at least in part, to the presence of fiber associated substances such as phytate and lectins which are present in feeds, and to a potential interaction between different fiber fractions (FAO, 1998). Bach Knudsen and Hansen (1991) reported that when soluble and insoluble NSP content of the diet increased from 20 and 14 g/kg to 52 and 44 g/kg, respectively, the ileal and total tract apparent digestibility of protein decreased dramatically (0.14 and 0.13 unit, respectively). Comparing the effect of soluble and insoluble NSP on the digestibility of protein, Robertfroid (1993) reported that soluble NSP (like pectin) were expected to have a larger negative effect than insoluble NSP (NDF). However, quantitative relationships between different types of fiber and protein digestibility were not given to support that idea.

**Fiber-protein interaction**

It has been demonstrated that different types of fiber can influence the digestibility of protein and amino acids. However, it is also interesting to consider whether dietary protein level interferes with the effect of fiber on the N digestibility. Investigation of a potential interaction of fiber and protein on protein digestibility is crucial considering the strong correlation between ileal digestible protein and/or amino acid and pig performance (average daily gain, feed conversion and protein deposition). Dietary protein and fiber did not interact for N excretion variables or N-retention, therefore the effect was suggested to be additive in the study of Zervas and Zijlstra (2002a and b). Fan and Sauer (2002) also did not find an interaction between NDF and protein or amino acid intake with regard to apparent
protein and amino acid digestibility. The additive effects of both protein and dietary fiber are supported by ammonia emission study as well; lowering dietary protein reduced gaseous ammonia emission and also the increasing fermentable fiber, and the combination of protein and fiber reduced ammonia emission additionally (Kreutzer et al., 1998).

It is clear from the literature that increasing dietary NDF increases the endogenous N and amino acid losses (Schultze et al., 1994; Leterme et al. 1996; Lenis et al. 1996). Theoretically, whether dietary fiber had no other effect than enhancing the endogenous N excretion, the apparent ileal digestibility should be reduced with increasing dietary fiber content and this must be more pronounced when low protein diets are fed (Fan et al. 1994; Stein at al. 2007).

Reviewing the literature it can be stated that only a few studies have been conducted in which different dietary fiber levels with different protein and/or amino acid levels were used. Moreover, since the aim of the trials were not actually to study the effect of fiber-protein interaction, the design of the experiments might be inappropriate to measure this interaction in most cases due to the small increments or few steps in the dietary treatment.

3.4. Effect of dietary fiber on ileal endogenous nitrogen losses

The ileal endogenous amino acid losses are endogenously synthesized proteins secreted into the intestinal lumen that has not been digested and reabsorbed before reaching the distal ileum (Hodkinson and Mougham, 2000). Endogenous proteins consist of salivary and gastric secretions, pancreatic and bile secretions, small intestinal secretions, mucus, sloughed epithelial cells and microbial protein (Jansman 2002). Mucin is very rich in threonine up to 30 % of their of their amino acid composition (Neutra and Forstner, 1987), and considering that mucin is the main constituent of the
endogenous protein excretion that may explain the generally reported low 62 -73 % apparent digestibility of threonine (Huang et al.1999). Glycine is the main constituent base of bile salt, representing more than 90 % in the total amino acid secreted in porcine bile juice (Souffrant, 2001). Bile salts are conjugated in the large intestine and/or metabolized in particular the presence of fermentable fiber as effect of the microbial enzymes. Conjugated bile salts are reabsorbed and enter to the enterohepatic circulation meanwhile deconjugated glycine escapes reabsorption and fluxes to the large intestine (Newsholme and Leech, 1988)

**Determination of the ileal endogenous amino acid loss**

In the course of determination of the endogenous amino acid loss one of the most commonly used techniques to collect ileal digesta is the post-valve T caecum (PVTC) canulation, although simple T canulation is also satisfactory (Hodkinson and Mougham, 2000). The impact of removing the caecum (which occurs with PVTC canulation) on the animal remains to be fully determined.

Traditionally, a protein-free diet is used to determine the endogenous ileal amino acid flow but several alternative method have been developed to elucidate the effect protein supply on endogenous ileal amino acid loss. (Hodkinson, and Mougham, 2000; Jansman, 2002).

If the animals are fed with a protein free diet, than it is assumed that N and amino acids found in the digesta are of endogenous origin. It was reported that in case of protein-free method the prolin, but no other amino acid loss increases (de Lange et al., 1989; Sauer and de Lange, 1992). In a study de Lange et al. (1989) used N-free diet with or without intravenous
balanced amino acid infusion and confirmed that the negative N-balance has no significant influence on the amino acid excretion into the intestinal lumen. The method is broadly accepted and most frequently used to determine the endogenous N loss (Jansman et al., 2002).

Another approach to determine the endogenous amino acid flow is to feed fully digestible protein diets. For that purpose casein (or casein and gluten), semi-synthetic diets with purified amino acids or enzymatically hydrolyzed casein are used as the protein source. In theory, all amino acids are digested and the amino acids found in the ileal digesta must be of endogenous origin. However, untreated casein is not fully digested and 2-3% of the dietary amino acids are still found in digesta taken from the end of ileum (Rutherfurd and Moughan, 2003). The mentioned methods (semi-synthetic diets with purified amino acids and enzymatically hydrolyzed casein) are rarely used in practice (Jansman et al., 2002). A further method is the isotope dilution technique, but this needs specific and expensive infrastructure, and therefore it is not commonly used in practice. A final method is the so called linear regression method, when different levels of protein is fed and the endogenous loss is estimated by extrapolation of ileal amino acid flow to zero dietary protein intake.

The method relies upon mathematical extrapolation, which is always hazardous and consequently the estimates of endogenous loss often have very substantial errors of estimation (Hodkinson and Mougham, 2000).

Factors influencing the endogenous AA loss

Although the method of determination affects the endogenous N losses, the literature is consistent in concluding that the output of endogenous protein is mainly influenced by body weight, feed intake, presence of
antinutritional factors, and dry matter, protein and dietary fiber content of
the feed (reviewed by Souffrant, 2001). However, according to Sauer and
Ozimek (1986), the level and the source of dietary fiber are the two most
important factors influencing the amount of endogenous N present in the
ileal digesta. Inclusion of fiber increases the sloughing effect of intestinal
mucosal cells (Bergner et al. 1975) and enhances the mucus production
(Schneeman et al., 1982). There are examples that due to the exaggerated
endogenous protein losses the threonine can become limiting for pig growth
(Zhu et al., 2005). It is frequently reported in the literature that dietary fiber
or NDF linearly increases the endogenous N loss (Sauer et al., 1991;
Schultze et al., 1994; Lenis et al., 1996; Yin et al., 2000), however, some
studies show that from certain fiber content the endogenous amino acid loss
does not increase further (Taverner et al., 1981; Mariscal-Landin et al.,
1995). Due to physico-chemical properties of various fibers, soluble and
insoluble dietary fibers affect the endogenous protein losses differently. The
insoluble NDF stimulated the pancreatic digestive enzymes (Langlois et al.,
1987), while pectin, which is a soluble fiber, had no influence on pancreatic
juice and enzyme secretion (nor protease enzyme activity) in the study of
Mosenthin et al. (1994). Nevertheless the protein loss from the epithelium of
the gastrointestinal tract must be significant in the case of a diet containing
high amounts of soluble fiber due to the increased viscosity, as confirmed
by several studies (de Lange et al., 1989; Leterme et al, 1996; 1998).
Whereas the addition of pectin increased endogenous N flow, the amount of
endogenous N recovered in ileal digesta tended to increase with
supplementation of pure cellulose (de Lange et al., 1989). Although many
studies report an increasing endogenous protein loss with increasing fiber
content of the diet, Mosenthin et al. (1994) and Grala et al. (1998) suggested
that the reabsorption rate of endogenous N may be reduced in the small
intestine rather than only an increase in the amount of N loss. In a study of Libao-Mercado et al. (2006) inclusion of 22.5 or 45 % wheat short in the corn starch based diet reduced ileal digestibility of most amino acids (P<0.01). The effect of wheat shorts on endogenous amino acid losses (EAAL) was largely attributed to the soluble NSP content in wheat short and seemed to be non-linear (Libao-Mercado at al., 2006). Data of Myrie et al. (2008) suggest that hemicellulose fiber, at concentrations typical in commercial swine diets, reduces the ileal digestibility of AAs by increasing endogenous losses. However, those authors concluded also that understanding the differential effects of antinutritional factors on endogenous losses of individual dietary AA will improve the accuracy of diet formulation (Myrie et al., 2008).

**Apparent, true and standardized ileal digestibility**

Endogenous amino acid losses may be divided into two main components: basal and specific losses (Séve and Henry, 1996; Nyachoti et al. 1997a; Jansman et al., 2002; Stein et al., 2007). The basal losses as a nonspecific ingredient or diet independent losses represent the minimum inevitable lost by the animal. These losses are related to the physical flow of the dry mater and are not influenced by dietary composition (Figure 2). The specific losses above basal losses are ingredient specific and depend particularly on dietary fiber content and fiber type (Schulze et al. 1995) as well as influenced by antinutritional factors (Myrie et al., 2008).
Figure 2. Partitioning of AA in ileal digesta from pigs as influenced by dietary AA [Stein et al., (2007); adapted from Krawielitzki et al. (1977) and Fan et al. (1994)].

The amount of endogenous nitrogen losses (ENL) is required to determine the true protein and amino acid digestibility from the apparent digestibility. Apparent ileal digestibility (AID) of amino acids (AA) is determined as follows:

\[ \text{AID, } \% = \frac{[(\text{AA intake} - \text{Ileal AA outflow})/\text{AA intake}]}{100} \]

True ileal digestibility (TID) of AA is calculated from apparent ileal digestibility by making correction for the ileal endogenous AA losses:

\[ \text{TID, } \% = \frac{[(\text{AA intake} - (\text{ileal AA outflow} - \text{total IAAend}))/\text{AA intake}]}{100} \]

True digestibility has the advantage over apparent digestibility in that it is a fundamental property of the feedstuff, being independent of dietary conditions (Mosenthin, 2002). However, no sufficient information is available on the amount of specific and thus on total endogenous amino acid loss for different feedstuffs (Stein et al., 2007) considering that the determination of the endogenous loss is very complicated. Therefore, a so-called standardized protein and/or amino acid digestibility has been used in pig nutrition that accounts for only a basic endogenous N or amino acid excretion (Degussa, 2002), and therefore it is independent of dietary amino
acid level. Standardized ileal digestibility (SID) of AA is computed similarly as TID, but the correction is made only for basal IAAend:

\[
\text{SID, } \% = \left\{\frac{(\text{AA intake} - (\text{ileal AA outflow} - \text{basal IAAend}))}{\text{AA intake}}\right\} \times 100
\]

The specific ENL – above basal ENL – which is variable and related to the presence of inherent factors in the feedstuff such as fiber and antinutritional factors is ignored by using standardized protein digestibility. Due to the fact that only the basal ileal endogenous amino acid losses are subtracted from the total ileal AA outflow, values for SID are intermediate between values for AID and TID and independent of dietary AA level. The main advantage if using SID compared with AID is that values for SID are more likely to be additive in mixed diets (Stein et al., 2005). Apparent ileal digestibility is not additive and largely affected by the dietary AA level as is shown in Figure 3.

![Figure 3. Influence of the dietary AA content on the measured values of apparent, standardized, and true ileal AA digestibility [Stein et al., (2007); adapted from Krawielitzki et al. (1977) and Fan et al. (1994)].](image-url)
Reviewing the literature, it is clear that ileal digestibility of dietary protein is depressed by the presence of dietary fiber. It has also been reported, that the soluble NSP reduces the protein digestibility to a larger extent than insoluble NDF. Dietary fiber level influences the endogenous protein loss either by enhancing the excretion itself and/or by reducing its reabsorption. For that reason it is recommended to use preferentially standardized or true ileal digestibility data in diet formulation rather than apparent protein and/or amino acid digestibility when fiber rich ingredients are used extensively.

3.5 Effect of dietary fiber on fat digestibility

In practice, fibrous diet components dilute the nutrients in feed, therefore a high fiber pig diet is usually supplemented by fat or oil to compensate for energy dilution. The reduction of the digestibility of nutrients and particularly fat digestibility due to fiber has been reported frequently in the literature. Some studies addressing the effect of dietary fiber on fat digestibility are presented in Table 2. As it was shown, the mechanism of the effect of dietary fiber on digestion depends on the solubility of the fiber. Soluble NSP depresses the digestibility of fat by means of changing the viscosity of the digesta. Pasquier et al. (1996) found that the extent of in vitro lipolysis with gastric and pancreatic lipase was significantly decreased by emulsion prepared in the presence of high viscosity guar gum when compared with those obtained without fiber or with low or medium viscosity guar gum. Insoluble NSP reduces the transit time of the digesta in the total tract due to the faster flow in the hindgut and may result in a shorter time for digestive enzyme action, with particular concern for lipase. However, the passage time in the small intestine either is
not affected or even prolonged (Kesting et al., 1991); therefore it raises the question whether insoluble fiber interferes with the ileal fat digestibility. Högberg and Lindberg (2004) found that the ileal digestibility of fat did not change when the insoluble: soluble dietary fiber ratio changed between 7.3 and 2.4.

The reduction in apparent fecal digestibility of fat with an increase in dietary fiber can be further explained by a greater bacterial fat synthesis (i.e. fatty acid incorporation into bacteria) in the hindgut, as suggested by Dierick et al. (1990) and Le Goff and Noblet (2001). This idea is supported by the data of Table 2 as well, considering that while the total tract digestibility of fat was depressed in almost all studies, the reducing effect of fiber was not as obvious when digestibility was measured in the small intestine. Moreover, Mroz et al. (1996) showed that fecal digestibility of fat was decreased, while ileal digestibility of fat did not change when pigs were fed diets supplemented by soyhulls or cellulose as compared to pigs fed a corn based diet. It was also reported that certain fibrous constituents absorb sterolic derivates like bile acids in the digesta, thus preventing absorption and enhancing fecal excretion of these derivates as reviewed by Kreuzer et al. (2002). This mechanism may also explain the decreased fat digestibility, because of less emulgation in the small intestine due to the binding of bile acid. A consistent observation in rats is that soluble fibers increase the fecal bile acid excretion to a greater extent than do insoluble fibers (Anderson et al., 1994). It would suggest that soluble fiber had a larger impact on the fat digestibility than insoluble NDF. However, in a recent study, Högberg and Lindberg (2004) found that the increase in the solubility of dietary fiber significantly increased the total tract digestibility of fat.
### Table 2. Literary data on the effect of different type of fibers on digestibility of crude fat

<table>
<thead>
<tr>
<th>Authors</th>
<th>Fiber source</th>
<th>Body weight</th>
<th>Fiber type</th>
<th>Effect of fiber</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freire et al., 1998</td>
<td>wheat bran</td>
<td>5 kg</td>
<td>NDF 100 vs. 160 g/kg</td>
<td>faecal digestibility ↓</td>
</tr>
<tr>
<td>Le Goff and Noblet, 2001</td>
<td>77 different diets</td>
<td>61 kg and 234 kg</td>
<td>NDF 112-394 g/kg</td>
<td>faecal digestibility ↓</td>
</tr>
<tr>
<td>Noblet and Perez, 1993</td>
<td>114 different diets</td>
<td>45 kg</td>
<td>NDF 44-261 g/kg</td>
<td>faecal digestibility ↓</td>
</tr>
<tr>
<td>Wilfart et al., 2007</td>
<td>wheat by-products</td>
<td>33 kg</td>
<td>NDF 146-238 g/kg</td>
<td>faecal digestibility ↓</td>
</tr>
<tr>
<td>Bach Knudsen and Hansen, 1991</td>
<td>wheat by-products</td>
<td>40-50 kg</td>
<td>NSP 30-54 g/kg, lignin 4-8 g/kg</td>
<td>ileal dig. Ø or ↓; faecal dig. Ø</td>
</tr>
<tr>
<td></td>
<td>oat by-products</td>
<td>40-50 kg</td>
<td>NSP 34-96 g/kg, lignin 9-13 g/kg</td>
<td>ileal dig. ↓; faecal dig. ↓</td>
</tr>
<tr>
<td>Mroz et al., 1996</td>
<td>soybean hulls, cellulose</td>
<td>40 kg</td>
<td>NDF 87-355 g/kg, ADF 35-286 g/kg</td>
<td>ileal dig. Ø; faecal dig. ↓</td>
</tr>
<tr>
<td>Bakker et al., 1995</td>
<td>soyhulls, cellulose</td>
<td>60 kg and 90 kg</td>
<td>NDF 91-380 g/kg, CF 29-258 g/kg</td>
<td>faecal digestibility ↓</td>
</tr>
</tbody>
</table>

↓ - reducing effect, Ø - no effect
Hansen et al. (1991) showed that in case of low fat diets containing approximately 40 g/kg dietary fiber the assumption of additively of total digestibility of nutrients is correct. This might not be true for diets with large concentrations of fat and fermentable carbohydrate, because fermentable NSP and fat affect each other’s total tract digestibility (Bakker, 1996). Also, maize oil and type of fiber interact with fecal digestibility in rats (Key and Mathers, 1993). This interaction was confirmed in pigs by Bakker et al. (1995).

Bakker et al. (1995) reported that the measured ileal digestibility of fat was 0.04 and 0.07 units lower than expected when fat was added (70 g/kg) to soyhull and cellulose supplied diets (270 and 260 g/kg, respectively).

It can be concluded, that the effect of dietary fiber on fat digestibility has been studied extensively. Increasing dietary fiber decreases the fat digestibility or alternatively, increasing fat content reduces fermentation in the hind gut and results in a lower fiber digestibility. Recent results show that the detrimental effect of dietary fiber on fat digestibility is reduced by increasing the solubility of fiber. However, the effect of the interaction of NSP and fat on fat digestibility is not yet clear. In order to understand the interactive effect of dietary fiber levels and fat types, the mechanism governing fat digestion in the case of high dietary fiber needs to be resolved.

3.6 Consequences of fiber inclusion on diet formulations

Currently used energy evaluation systems in pig feeding, such as DE, ME and even NE, are based on digestible nutrients present in the diet. Indeed the differences between calculated and measured data are larger when fibrous ingredients are used in the pig diet. Thus, it emphasizes that the composition of the diet, particularly the fiber source and fiber type are also important factors in the energy properties of pig feeds. As it was shown
in the sections above, dietary fiber interferes with the digestibility of other nutrients and therefore the digestible nutrient content can be reduced. If the actual reduction experienced by the pig was smaller or larger than expected, a nutrient interaction would probably occur and has to be considered in diet formulations.

3.6.1 Relationship between dietary fiber content and digestible protein and amino acid content of the diet

It has been reviewed in earlier sections that digestibility of dietary protein and amino acids are reduced by dietary fibers. Since dietary fiber increases the endogenous protein loss, the apparent ileal digestibility of protein and amino acids are expected to be decreased more than true ileal digestibility. On the other hand, the increased endogenous protein losses may result in a somewhat higher amino acid and energy requirements of the pig according to the higher protein synthesis rate in the intestine. That requirement might be quantified and considered in the diet formulation.

Numerous literary data report that dietary fiber reduces total tract and/or ileal digestibility of protein and amino acids. However, a quantitative description of the relationship between different fibers and protein and/or amino acids was addressed in a few studies. Noblet and Perez (1993), and Le Goff and Noblet (2001) investigated 114 and 77 different diets, respectively, and computed linear regression of digestible crude protein as a function of crude protein and NDF content of the feed. Although the data basis is considerable, the equations provide an estimation of digestible protein measured in the total tract of the intestine, but data on fecal and ileal digestibility are not interchangeable. Drochner et al. (2004) developed an equation for apparent ileal digestibility of crude protein based on different trials using 22 dosages of crude fiber in total. This relationship can be used
generally; nevertheless, considering that various types of fiber reduce the digestibility of protein with different extension, one would expect a more precise definition on the fiber source in the equations. Regression equations for true ileal digestibility of the indispensible amino acids related to soy hull level in the diet was given by Dilger et al. (2004), however, the range of the inclusion of soy hull was limited to 9%.

In conclusion, crude fibre gives value for a wide range of components, often termed “fibre” but which in fact act very differently in the gastrointestinal tract. Due to physico-chemical properties of various fibers, soluble and insoluble dietary fibers affect the endogenous protein losses differently and that should be taken into consideration in diet formulation. New and more precise regression equations are required to predict the effect of different types of dietary fiber on protein and amino acid digestibility.

3.6.2. Relationship between dietary fiber content and digestible fat and energy content of the diet

Considering that digestibility of starch is almost complete, the energy intake from starch will hardly change when different types of polysaccharides are presented in the pig feed (Bach-Knudsen and Hansen, 1991; Bakker, 1996). However, according to the high energy content of dietary fat, the effect of fiber on fat digestibility determines the DE content of the diet. It has been repeatedly reported that increasing dietary fiber decreases digestibility of fat. This effect was quantitatively described by Noblet and Perez (1993) and Le Goff and Noblet (2001). They found that the impact of the NDF fraction is significant, considering that each g of NDF per kg DM depresses the digestible fat content by 20-30 g/kg. This results in approximately 0.001 unit reduction per 1 g NDF/kg DM in the digestibility coefficient of energy. Considering that the range of NDF in
feed ingredients is from about 90 up to 850 g/kg (Johnston et al., 2003), this 0.001 unit decrease per 1 g/kg NDF is significant. On the other hand, as it was discussed above, the effect of different types of fiber on digestion has not been clarified. Based on previous sections one would expect a different response of fat digestibility if soluble vs. insoluble dietary fibers were fed. Therefore, considering the solubility and other characteristics of fiber, different equations for energy content are required in diet formulation.

As reported in some studies (Shi and Noblet, 1994; Bakker, 1996) interaction occurs between dietary fiber and fat, if both nutrients are presented in the diet at high concentrations. In the study of Bakker (1996) 70 g/kg additional animal fat resulted in a 2 and 5 % reduction in energy supply prior to the caecum, by combining fat in the diets with soyhulls (270 g/kg) and cellulose (260 g/kg), respectively. This suggests that even the energy supply from fat or rather the energy supply from fiber was overestimated. The latter is feasible since high fat diets reduce the fermentation in the hindgut. In contrast, Shi and Noblet (1994) found a reverse effect; the combination of rapeseed oil and a ‘fiber-mixture’ (wheat bran, soya bean hulls, sugar beet pulp and wheat straw) resulted in a higher measured ileal DE supply than was calculated. The difference between the two results may be explained by either vegetable oil vs. animal fat or pure vs. 'mixed’ fiber being used. This interaction, however, modifies the effect of fiber on fat digestibility and thus, the DE content of the diet becomes roughly predictable.

Högberg and Lindberg (2004) found that fat and energy digestibility increased when the solubility of fiber increased. In their study, both soluble and insoluble fibers were presented in the diets. Bach Knudsen (2001) reported that soluble dietary fiber did not change the fecal digestibility of energy. It seems soluble fiber has no effect on energy digestibility due to
fermentation in the hind gut the produced volatile fatty acids being utilized as an energy source by the animals. However, insoluble fiber has a negative effect on energy digestibility but it is not clear how this effect is modified by fat supplementation.

In recent years two principles are widely accepted in pig nutrition; firstly using an optimal ileal digestible lysine/DE ratio, and secondly formulating an ‘ideal protein’ ratio (Close, 1994; NRC, 1998). For the most efficient utilization of feed, the most appropriate feeding strategy is one which meets exactly the nutrient requirements of the pig (Close, 1994). Even a small deviation from the optimal ileal digestible lysine/DE ratio results in undesirable performance including poorer feed conversion and increasing fat content in the carcass. Therefore, it is crucial to determine the DE content of the pig feed with certainty and accuracy, otherwise the ileal digestible lysine/DE ratio fails. The ideal protein concept suggests an optimal ratio of amino acids, in which all indispensable amino acids are related to lysine. It has been repeatedly shown that feeding diets with fiber rich ingredients, results in decreased digestibility of protein and amino acids. Therefore quantifying the effect of fiber, considering its solubility and the interaction between dietary fiber and fat as well as the interaction between fiber and protein on digestive processes, are strongly recommended.

3.7 Conclusions from the literature

The behavior of different types of dietary fibers in the digestive processes depends in particular on their physico-chemical properties such as solubility and viscosity. Literary data show that soluble fiber reduces the apparent and true ileal digestibility of protein and amino acids more than insoluble fibers; however, fecal digestibility of energy is compromised by
insoluble rather than soluble fiber content of the diet. It is necessary to develop regression equations that compute the reduction in ileal digestibility of nutrients as a function of different types of fiber, when fiber rich ingredients are used in diet formulation. Due to fiber-fat interactions, there may be significant differences between calculated and measured energy content of pig diets in the case of high fiber and high fat content. In order to do a precise and accurate diet formulation of a pig diet, further studies are required for the quantification of the effect of fiber-fat interaction on the digestible nutrient supply.
4. Aim of the study
The following objectives were determined:

1) to evaluate and quantify the effect of different dietary fiber and fat level on the total tract digestibility of nutrients and gross energy.

2) to determine the effect of increasing fiber content from different sources (from wheat bran and soy hulls) on the SID of selected amino acids (lysine, methionine, cystine threonine, and tryptophan).

3) to develop regression equations that can be used to predict the SID of selected amino acids applicable for different fiber sources commonly used in hog.

4) to obtain essential data for application in practical diet formulation when fiber rich components are used.
5. Materials and methods
5.1 Animals and housing

To accomplish the aim of the study 2 trials were carried out at Department of Animal Nutrition in Kaposvár University, Hungary. The experiments were approved by the Ethical Committee of Kaposvár University, Hungary.

5.1.1 Trial 1 (Total tract digestibility study)

A total of 125 castrated hybrid (Large White x Landrace) growing pigs, 5 animals/treatment with no replicates with an initial body weight of 39 kg were used in the serial trial. The experiment was carried out with 25 dietary treatments. The animals were kept individually during the digestibility trials in metabolic cage. The room temperature and relative humidity were regulated in accordance with the requirements of growing pigs (NRC, 1998).

5.1.2 Trial 2 (Ileal digestibility study)

The trials were conducted with total of 40 castrated hybrid (Large White x Landrace) growing pigs, 4 hybrid barrows per treatment, in 2 replicates (8 animals/treatment), with an initial mean live weight of 30 kg. Before starting the trials the animals were fitted with PVTC-cannula (Figure 4 and 5). The surgical operations were performed in accordance with van Leeuwen et al. (1991). With this surgery operation a specially shaped T-cannula is sucked into the cannula by vacuum generated and with the control of ileo-caecal valve the entire digesta is voided outside. A major advantage of the method is that the necessary operation is relatively simple, the peristalsis of the small intestine is undistributed and it allows quantitative collection (Babinszky, 2008). Before the surgical operation, during the recovering and adaptation period of the trials the animals were
kept in special individual pens, while in the collection phase of the trial they were kept in metabolic cages designed for growing pigs. The room temperature and relative humidity were regulated in accordance with the requirements of growing pigs (NRC, 1998).

Figure 4. Post valve T-canulation technique in pigs

Figure 5. The fitted PVTC canula after surgery
5.2 Diets and feeding

5.2.1 Trial 1 (Total tract digestibility study)

The experiment was carried out with 25 dietary treatments. Pigs were fed with diets based on five different wheat bran levels (0, 150, 300, 450 and 600 g/kg) and five added fat levels (0, 25, 50, 75 and 100 g/kg) in a 5 x 5 factorial arrangement (Table 3). The dietary fat was a mix of animal fat and vegetable oil in which the unsaturated/saturated fatty acid (U/S) ratio was 1.74 (SAT: 36.4 %, MUFA: 47.1 %, PUFA : 16.5 %). The composition and nutrient content of the diets are presented in Table 4. The daily DE intake was adjusted as 2.6 times of the maintenance requirement (DE for maintenance was assumed as 460 kJ/kg^{0.75}/day according to NRC, 1998). The daily nutrient intake presented in Table 5. Due to the different energy content of the feeds, the daily feed intake was different, but the energy intake and the ileal digestible protein, lysine, methionine, cystine and threonine intake were the same in each treatment. The experimental animals were fed twice daily and were allowed free access to water.

Table 3. Arrangement of the trial

<table>
<thead>
<tr>
<th>Wheat bran (WB) Content of the diet (%)</th>
<th>Added fat (AF) %</th>
<th>0</th>
<th>2.5</th>
<th>5</th>
<th>7.5</th>
<th>10</th>
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</thead>
<tbody>
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<tr>
<td>AF 0</td>
<td></td>
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<td>AF 5</td>
<td>AF 7.5</td>
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<tr>
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<td>AF 7.5</td>
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<td>AF 5</td>
<td>AF 10</td>
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<td>Ingredients</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<td>1000.0</td>
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</tbody>
</table>

**Nutrient content**

- **DE (MJ/kg)**
- **ME (MJ/kg)**
- **TID lysine**
- **TID Met+Cys**
- **Crude protein**
- **Crude fat**
- **Crude fiber**
- **Ca**
- **P**

1: calculated data  
TID: true ileal digestible  
Table 5. Daily nutrient intake of a 40 kg live weight pig in Trial 1. Model calculation based on table 4 values and NRC (1998)

<table>
<thead>
<tr>
<th>Analyzed nutrients</th>
<th>Wheat bran 0 g/kg</th>
<th>Wheat bran 150 g/kg</th>
<th>Wheat bran 300 g/kg</th>
<th>Wheat bran 450 g/kg</th>
<th>Wheat bran 600 g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Added fat (g/kg)</td>
<td>Added fat (g/kg)</td>
<td>Added fat (g/kg)</td>
<td>Added fat (g/kg)</td>
<td>Added fat (g/kg)</td>
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<tr>
<td></td>
<td>0 25 50 75 100</td>
<td>0 25 50 75 100</td>
<td>0 25 50 75 100</td>
<td>0 25 50 75 100</td>
<td>0 25 50 75 100</td>
</tr>
</tbody>
</table>

* Calculated nutrients
5.2.2 Trial 2 (Ileal digestibility study)

5.2.2.1. Pilot study on endogenous amino acid excretion

The rate of ileal endogenous protein and amino acid excretion were studied in a total of four treatments. The basal N-free diet was supplemented with different levels of cellulose (Arbocell, produced by J. Rettenmaier and Söhne): 30-, 55-, 80 and 105 g/kg). The composition and the nutrient content of the experimental diets are given in Table 6.

5.2.2.2 Standardized Ileal Digestibility study with wheat bran and soy hulls

In the digestibility study 10 dietary treatments were used with increasing wheat bran (WB) or soyhulls (SH) inclusion rates. The basal diet was a corn-soybean meal diet that was supplemented with 0, 25, 50, 75 and 100 g/kg wheat bran (WB-0, WB-25, WB-50, WB-75, WB-100, respectively) or soyhulls (SH-0, SH-25, SH-50, SH-75, SH-100, respectively). The analyzed NDF content of the feeds ranged from 135 to 167 g/kg in the wheat bran diets and from 135 to 179 g/kg in the soyhulls diets. The composition and nutrient content of the experimental diets are given in Tables 7 and 8. The amino acid content of the experimental diets is shown in Tables 9 and 10. The daily nutrient intake is presented in Tables 11 and 12.
Table 6. Composition and nutrient content of the N-free diets (g/kg)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>T</th>
<th>R</th>
<th>E</th>
<th>A</th>
<th>T</th>
<th>M</th>
<th>E</th>
<th>N</th>
<th>T</th>
<th>S</th>
</tr>
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<tbody>
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<tr>
<td>Sacharose</td>
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<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
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<td></td>
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<tr>
<td>Cellulose (Arbocell)(^1)</td>
<td>30.00</td>
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<td></td>
</tr>
<tr>
<td>Fat (vegetable)</td>
<td>30.00</td>
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<td>52.00</td>
<td>62.00</td>
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<td></td>
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<tr>
<td>MCP (^2)</td>
<td>22.50</td>
<td>22.50</td>
<td>22.50</td>
<td>22.50</td>
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<td>5.10</td>
<td>5.10</td>
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<td>Vit.-min. Premix(^3)</td>
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**Nutrient content**

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<th>A</th>
<th>T</th>
<th>M</th>
<th>E</th>
<th>N</th>
<th>T</th>
<th>S</th>
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<tbody>
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<tr>
<td>DEs(^4) (MJ/kg)</td>
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<td>15.7</td>
<td>15.7</td>
<td>15.7</td>
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<td></td>
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</tr>
<tr>
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<td>15.1</td>
<td>15.1</td>
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<td></td>
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<tr>
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1. Crude fiber content: 65 % cellulose produced by J. Rettenmaier and Söhne
2. Ca165 /P2163

---

4. calculated value
Table 7. Composition of the experimental diets formulated with wheat bran inclusion (g/kg)

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<th>T</th>
<th>M</th>
<th>E</th>
<th>N</th>
<th>T</th>
<th>S</th>
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<td>198.00</td>
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<table>
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<th></th>
</tr>
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<td>159.0</td>
<td>162.0</td>
<td>160.0</td>
</tr>
<tr>
<td>Crude fat</td>
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<td>70.0</td>
<td>67.0</td>
<td>72.0</td>
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** : calculated value
Table 8. Composition of the experimental diets formulated with soy hulls inclusion (g/kg)

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<th>R</th>
<th>E</th>
<th>A</th>
<th>M</th>
<th>E</th>
<th>N</th>
<th>T</th>
<th>S</th>
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<td>Soybean meal (CP48 %)</td>
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<td>202.00</td>
<td>192.00</td>
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<td>180.00</td>
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<td>NaCl</td>
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<tr>
<td>Lysine-HCl</td>
<td>2.78</td>
<td>2.69</td>
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<tr>
<td>DL-methionine</td>
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<td>0.47</td>
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<td>0.56</td>
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</tr>
<tr>
<td>L-threonine</td>
<td>0.49</td>
<td>0.49</td>
<td>0.53</td>
<td>0.51</td>
<td>0.54</td>
<td></td>
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</tr>
<tr>
<td>L-Tryptophan</td>
<td>0.09</td>
<td>0.10</td>
<td>0.12</td>
<td>0.12</td>
<td>0.14</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Vit.-min. Premix*</td>
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<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cr2O3</td>
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<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>1000.00</td>
<td>1000.00</td>
<td>1000.00</td>
<td>1000.00</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Nutrient content

- Dry matter: 898.0, 896.0, 896.0, 897.0, 898.0
- DEs** (MJ/kg): 15.0, 14.9, 14.7, 14.6, 14.4
- MEs** (MJ/kg): 14.4, 14.3, 14.1, 14.0, 13.8
- Crude protein: 166, 164, 160, 162, 157
- Crude fat: 61, 62, 61, 64, 65
- Crude fiber: 19, 28, 37, 43, 54
- Crude ash: 50, 51, 49, 48, 49
- N-free extract: 602, 591, 589, 580, 573

**NDF**: 135, 148, 159, 167, 179

- ADF: 62, 72, 81, 89, 99
- ID LYS**: 8.2, 8.1, 8.1, 8, 7.9
- ID M+C**: 4.7, 4.6, 4.6, 4.5, 4.5
- ID THR**: 4.9, 4.9, 4.8, 4.8, 4.7
- ID TRP**: 1.4, 1.4, 1.4, 1.4, 1.4
- Ca: 6.6, 7, 6.6, 6.7, 6.6
- P: 6.1, 5.9, 5.8, 6, 5.8


** calculated value
Table 9. Amino acid content of the experimental diets formulated with wheat bran inclusion (g/kg), measured values

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>T</th>
<th>R</th>
<th>E</th>
<th>A</th>
<th>T</th>
<th>M</th>
<th>E</th>
<th>N</th>
<th>T</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WB-0</td>
<td>WB-25</td>
<td>WB-50</td>
<td>WB-75</td>
<td>WB-100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lysine</td>
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<td>9.8</td>
<td>9.7</td>
<td>9.8</td>
<td></td>
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<tr>
<td>Methionine</td>
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<td>3.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cystine</td>
<td>2.9</td>
<td>2.9</td>
<td>2.9</td>
<td>2.9</td>
<td>2.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methionine+Cystine</td>
<td>6.0</td>
<td>6.0</td>
<td>5.9</td>
<td>5.9</td>
<td>5.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Threonine</td>
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<td>6.6</td>
<td>6.5</td>
<td>6.5</td>
<td>6.5</td>
<td></td>
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<tr>
<td>Tryptophan</td>
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</tr>
<tr>
<td>Arginine</td>
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<td>10.0</td>
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<td>9.8</td>
<td>9.9</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Isoleucine</td>
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<td>6.3</td>
<td>6.2</td>
<td>6.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valine</td>
<td>7.6</td>
<td>7.5</td>
<td>7.4</td>
<td>7.3</td>
<td>7.4</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Table 10. Amino acid content of the experimental diets formulated with soyhulls inclusion (g/kg), measured values

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>T</th>
<th>R</th>
<th>E</th>
<th>A</th>
<th>T</th>
<th>M</th>
<th>E</th>
<th>N</th>
<th>T</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SBH-0</td>
<td>SBH-25</td>
<td>SBH-50</td>
<td>SBH-75</td>
<td>SBH-100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lysine</td>
<td>10.0</td>
<td>9.8</td>
<td>9.7</td>
<td>9.6</td>
<td>9.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methionine</td>
<td>3.1</td>
<td>3.1</td>
<td>3.2</td>
<td>3.1</td>
<td>3.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cystine</td>
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<td>2.9</td>
<td>3.0</td>
<td>2.8</td>
<td>2.8</td>
<td></td>
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</tr>
<tr>
<td>Methionine+Cystine</td>
<td>6.0</td>
<td>6.0</td>
<td>6.2</td>
<td>5.9</td>
<td>5.9</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Threonine</td>
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<td>6.5</td>
<td>6.4</td>
<td>6.4</td>
<td>6.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tryptophan</td>
<td>1.9</td>
<td>1.9</td>
<td>1.9</td>
<td>1.8</td>
<td>1.8</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Arginine</td>
<td>10.1</td>
<td>9.9</td>
<td>9.7</td>
<td>9.6</td>
<td>9.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoleucine</td>
<td>6.5</td>
<td>6.5</td>
<td>6.3</td>
<td>6.3</td>
<td>6.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valine</td>
<td>7.6</td>
<td>7.5</td>
<td>7.4</td>
<td>7.3</td>
<td>7.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
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</table>
Table 11. Daily nutrient intake of a 30 kg live weight pig fed diets with different wheat bran level in Trial 2

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>WB-0</th>
<th>WB-25</th>
<th>WB-50</th>
<th>WB-75</th>
<th>WB-100</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEs* (MJ/kg)</td>
<td>15.3</td>
<td>15.3</td>
<td>15.3</td>
<td>15.3</td>
<td>15.3</td>
</tr>
<tr>
<td>Crude protein (g)</td>
<td>169</td>
<td>166</td>
<td>164</td>
<td>169</td>
<td>168</td>
</tr>
<tr>
<td>Crude fat (g)</td>
<td>62</td>
<td>71</td>
<td>72</td>
<td>69</td>
<td>75</td>
</tr>
<tr>
<td>Crude fiber (g)</td>
<td>19</td>
<td>24</td>
<td>28</td>
<td>29</td>
<td>33</td>
</tr>
<tr>
<td>NDF (g)</td>
<td>138</td>
<td>146</td>
<td>157</td>
<td>167</td>
<td>175</td>
</tr>
<tr>
<td>ADF (g)</td>
<td>63</td>
<td>65</td>
<td>69</td>
<td>72</td>
<td>74</td>
</tr>
<tr>
<td>ID LYS* (g)</td>
<td>8.3</td>
<td>8.3</td>
<td>8.2</td>
<td>8.3</td>
<td>8.3</td>
</tr>
<tr>
<td>ID M+C* (g)</td>
<td>4.8</td>
<td>4.7</td>
<td>4.7</td>
<td>4.8</td>
<td>4.7</td>
</tr>
<tr>
<td>ID THR* (g)</td>
<td>5.0</td>
<td>4.9</td>
<td>4.9</td>
<td>5.0</td>
<td>4.9</td>
</tr>
<tr>
<td>ID TRP* (g)</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
</tr>
</tbody>
</table>

* Calculated nutrients

Table 12. Daily nutrient intake of a 30 kg live weight pig fed diets with different soyhulls level in Trial 2

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>SBH-0</th>
<th>SBH-25</th>
<th>SBH-50</th>
<th>SBH-75</th>
<th>SBH-100</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEs* (MJ/kg)</td>
<td>15.3</td>
<td>15.3</td>
<td>15.3</td>
<td>15.3</td>
<td>15.3</td>
</tr>
<tr>
<td>Crude protein (g)</td>
<td>169</td>
<td>168</td>
<td>167</td>
<td>170</td>
<td>167</td>
</tr>
<tr>
<td>Crude fat (g)</td>
<td>62</td>
<td>63</td>
<td>63</td>
<td>67</td>
<td>69</td>
</tr>
<tr>
<td>Crude fiber (g)</td>
<td>19</td>
<td>28</td>
<td>38</td>
<td>45</td>
<td>57</td>
</tr>
<tr>
<td>NDF (g)</td>
<td>138</td>
<td>152</td>
<td>166</td>
<td>175</td>
<td>190</td>
</tr>
<tr>
<td>ADF (g)</td>
<td>63</td>
<td>74</td>
<td>84</td>
<td>93</td>
<td>105</td>
</tr>
<tr>
<td>ID LYS* (g)</td>
<td>8.4</td>
<td>8.3</td>
<td>8.5</td>
<td>8.4</td>
<td>8.4</td>
</tr>
<tr>
<td>ID M+C* (g)</td>
<td>4.8</td>
<td>4.7</td>
<td>4.8</td>
<td>4.7</td>
<td>4.8</td>
</tr>
<tr>
<td>ID THR* (g)</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>ID TRP* (g)</td>
<td>1.4</td>
<td>1.4</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
</tbody>
</table>

* Calculated nutrients
5.3 Experimental procedure

The schedules of the experimental procedure is summarized in Table 13.

Table 13. The schedules of the experimental procedure

<table>
<thead>
<tr>
<th>Items</th>
<th>Daily amount</th>
<th>Feeding time</th>
<th>Adaptation</th>
<th>Collection Day</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total tract digestibility</td>
<td>2.6 times maintenance NRC</td>
<td>8.00 am; 3.00 pm</td>
<td>9 days</td>
<td>5 days</td>
<td>24 h</td>
</tr>
<tr>
<td>Ileal dig. N.free</td>
<td>1.5 times maintenance NRC</td>
<td>8.00 am; 8.00 pm</td>
<td>5 days</td>
<td>3 days</td>
<td>12 h (8am – 8pm)</td>
</tr>
<tr>
<td>Ileal dig. WB and SH</td>
<td>2.6 times maintenance NRC</td>
<td>8.00 am; 8.00 pm</td>
<td>5 days</td>
<td>3 days</td>
<td>12 h (8am – 8pm)</td>
</tr>
</tbody>
</table>

5.3.1 Trial 1 (Total tract digestibility study)

A 9-day adaptation period was followed by a 5-day collection period, during which faeces were collected quantitatively. The animals were housed in metabolic cages during both the adaptation and collection periods. Pigs received their diet twice daily in mash form (1 part diet + 2 parts water) and were allowed free access to water. Feed refusals were collected and weighed daily. Fresh faeces production was weighed twice daily (at 8.00 a.m. and at 3.00 p.m.) and stored below -18°C until analysis. All the produced faeces were collected. At the end of the collection period the collected samples were homogenized, sub sampled and analyzed.
5.3.2 Trial 2 (Ileal digestibility study)

In the course of the trial the animals were fed the diets in mash form (1 part diet + 2 parts water). Their daily ration of the nitrogen-free diet was 1.5 times their maintenance energy requirement and that of the various wheat bran diets were 2.6 times their maintenance energy requirement. The feed was distributed in two equal parts and given at 8:00 a.m. and 20:00 p.m. The relatively long time between the two feedings was due to the digesta collection method. Drinking water was available to the animals as needed.

The trials consisted of a 5 day adaptation period and a 3 x 12 hours collection period. In the collection phases the total amount of the digesta was collected continuously (for 12 hours) according to the recommendations of Tanksley et al (1985). The volume of the collected digesta was weighed continuously and thereafter 30% of the total collected volume was freeze-dried after homogenization. The laboratory analysis was conducted with the samples so prepared. The live weights of the trial animals were recorded at the start of the adaptation period and at the start and end of the collection period.

5.4 Laboratory analysis

The nutrient (dry matter, crude protein, crude fat, fatty acids, crude fiber, N-free extract, crude ash and NDF) and amino acid content of the feeds and the amino acid content of digesta samples were determined according to AOAC (2000). The GE content of the diet and faeces was determined with adiabatic bomb calorimeter (IKA-C-4000). Prior to the experiment feed components were analyzed chemically and the formulation
of experimental diets were based on laboratory results. The remaining faeces were dried and ground (1 mm). Dry matter, crude fat, starch and reducing sugars of the faeces were determined in dried samples. Crude protein was analysed in fresh samples.

5.5 Calculations and statistical analysis

5.5.1 Trial 1 (Total tract digestibility study)

The calculation of digestible energy content (DE) of the feeds was done according to Schiemann et al. (1972) as follows:

\[
DE (MJ/kg) = (24.2 \times dP) + (39.4 \times dEE) + (18.4 \times dF) + (17.0 \times dNfe)
\]

where:

- \(dP\) : digestible crude protein (g/kg)
- \(dEE\) : digestible crude fat (g/kg)
- \(dF\) : digestible crude fiber (g/kg)
- \(dNfe\) : digestible N-free extract (g/kg)

**Statistical analysis**

The experimental data were evaluated by means of ANOVA (SAS, 2004). For determination of the mean effect on digestibility coefficients two-ways ANOVA was used with the following general model:

\[
Y_{ijk} = \mu + A_i + B_j + (AxB)_{ij} + e_{ijk}
\]

where:

- \(Y_{ijk}\) : dependent variables
- \(\mu\) : overall mean
- \(A_i\) : effect of fiber level, i=5 (0, 150, 300, 450 and 600 g/kg wheat bran)
- \(B_j\) : effect of fat level, i=5 (0, 25, 50, 75 and 100 g/kg of added fat)
- \((A \times B)_{ij}\) : interaction of fiber and fat level
- \(e_{ijk}\) : residual error
In the course of the statistical analysis the variance analyses showed significant interaction between fiber and fat effect. Therefore, the digestibility results are presented separately and group differences at P≤0.05 level tested by Tukey’s test (SAS, 2004).

The contribution of the main effects (fat, fiber, fat x fiber) to the variance of the dependent variables was calculated by the VARCOMP procedure of SAS (2004). The goal of VARCOMP procedure is to estimate the contribution of each of the random effects to the variance of the dependent variable. If the replication and/or treatment x replication (T_i x R_j) interaction were not significant, the effect of replication and/or interaction was omitted from the model.

Multiple linear regressions (PROC REG) were used to predict the digestibility of nutrients and energy as a function of crude protein, crude fat, crude fiber and N-free extract (SAS, 2004).

5.5.2 Trial 2 (Ileal digestibility study)

Standardized ileal digestibility (SID) of each amino acid was computed by the following equation, according to Jondreville et al. (1995):

\[
\text{SID} \% = \left( \frac{\text{AA intake [g]} - (\text{ileal AA outflow [g]} - \text{endogenous AA [g]})}{\text{AA intake [g]}} \right) \times 100
\]

Where according to experiment 2 the endogenous AA loss was assumed to be 406, 52, 103, 592 and 204 mg/kg dry matter intake for lysine, methionine, cystine, threonine and tryptophan, respectively.

There was no significant replication effect on the digestibility values. The experimental data was analyzed separately for treatments containing wheat bran and soyhulls by using a one-way ANOVA (SAS, 2004). In the
case of a significant treatment effect (P<0.05) the statistical reliance of differences among treatments was verified with Tukey test (SAS, 1999). The relationship between wheat bran and soyhulls level of the diet and standardized ileal digestibility of amino acids was examined with regression analysis separately in each fiber source (SAS, 2004). The following regression analyses were carried out:

- linear \( (Y = a_0 + a_1 X) \),
- quadratic \( (Y = a_0 + a_1 X + a_2 X^2) \) and
- linear-plateau with sharp transition \( (Y = a_0 - a_1^* \ln[1 + \exp(a_2 - X)] \);

where

\( X \) is level of wheat bran or soyhulls in the diet [g/kg] and
\( Y \) is SID of the amino acid [%]).
6. Results and discussion
6.1 The impact of dietary fiber and fat levels on total tract digestibility of energy and nutrients in growing pigs

In the present study, dietary wheat bran increased from 0 up to 600 g/kg (5 levels) combined with a wide range of supplemented dietary fat from 0 to 100 g/kg (5 levels). Obviously, many of the diets in the present 5 x 5 factorial design are far from normal feeding practice; however, extreme combinations are also required to quantify the magnitude of fiber x fat interaction. Accordingly the effect of dietary treatments on digestibility of nutrients (crude protein, crude fat, crude fiber and gross energy) Tables 14,16,17,18 respectively and is summarized in Table 15.

6.1.1 Digestibility of crude protein

There was no extreme data among digestibility coefficients of protein in different dietary treatments ranging between 80.5 and 89.5 % (Table 11).

Table 14. The effect of wheat bran (WB) and fat inclusion on total tract digestibility of crude protein (%)

<table>
<thead>
<tr>
<th>WB (%)</th>
<th>Added fat (%)</th>
<th>0.0</th>
<th>2.5</th>
<th>5.0</th>
<th>7.5</th>
<th>10.0</th>
<th>RMSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>89.5&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>87.8&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>87.6&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>87.0&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>87.7&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>85.7&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>85.5&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>87.5&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>86.5&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>86.7&lt;sup&gt;ABa&lt;/sup&gt;</td>
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<td></td>
</tr>
<tr>
<td>30</td>
<td>85.5&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>84.8&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>87.8&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>86.5&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>86.1&lt;sup&gt;ABa&lt;/sup&gt;</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>82.9&lt;sup&gt;BCab&lt;/sup&gt;</td>
<td>81.5&lt;sup&gt;Bb&lt;/sup&gt;</td>
<td>80.5&lt;sup&gt;Bb&lt;/sup&gt;</td>
<td>83.7&lt;sup&gt;Abab&lt;/sup&gt;</td>
<td>85.9&lt;sup&gt;ABa&lt;/sup&gt;</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>81.6&lt;sup&gt;Ca&lt;/sup&gt;</td>
<td>81.0&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>83.6&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>81.9&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>83.2&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>RMSE</td>
<td>1.9</td>
<td>2.1</td>
<td>1.6</td>
<td>2.4</td>
<td>2.0</td>
<td>1.8</td>
<td></td>
</tr>
</tbody>
</table>

<sup>ABCDE</sup> Within a column, means without a common superscript letter differ (P≤0.05)

<sup>abcde</sup> Within a row, means without a common superscript letter differ (P≤0.05)

RMSE Root mean square error
Fat supplementation had no impact on protein digestibility within the same wheat bran level except from diets with 450 g/kg of wheat bran. For those treatments there was significant difference among dietary treatments with different fat levels, however, we suppose that this significance was obtained by coincidence due to the small RMSE (0.9). Although, Li et al. (1994) found some improvement in ileal digestibility of protein and amino acids when the diet was supplemented rape oil (32-122 g/kg), the difference disappeared at the total tract level. Fat content of the diet did not improve the digestibility, however, comparing the protein digestibility data at 0 and 100 g/kg fat supplementation (ranges in 89.5-81.6 and 87.7-83.2 %, respectively), it might be noted that dietary fat slightly moderated the detrimental effect of wheat bran. It is probably due to the lubricant effect of fat that reduced the N loss by less desquamated cells.

Increased dietary wheat bran content resulted in significant reduction in protein digestibility (P<0.05). This observation is in agreement with several studies reporting that increasing dietary fiber has negative effect on protein digestibility (Lenis et al., 1996; Bach-Knudsen and Hansen, 1991; Yin et al., 2000; Libao-Mercado et al., 2006). There are several reasons that might explain the detrimental effect of wheat bran inclusion on protein digestibility:

(1) increasing passage rate of digesta through the entire gastrointestinal tract (Jorgensen et al., 1996; Le Goff et al., 2002; Morel et al., 2006) and thus shorter mean retention time reduces the efficiency of proteolytic enzyme action and the protein hydrolysis. Furthermore,
a higher endogenous N excretion with increasing wheat bran content could also explain, at least partly, the results found in this study (Furuya and Kaji, 1992; Schulze, 1994; Libao-Mercado et al., 2006). Last but not least, the reason for reduced protein digestibility by wheat bran inclusion could be that

(3) the N present in the NDF matrix is unavailable when it is not fermented (Schulze, 1994). Wheat bran contains 150 g/kg crude protein, however, it is partly bounded by NDF, which cannot be hydrolyzed by endogenous enzymes of the pig. With increasing wheat bran inclusion the proportion of NDF bound protein increased, resulting in reduced protein digestibility in the entire gastrointestinal tract. In dairy nutrition the NDF bound N – called neutral detergent insoluble protein (NDIP) – is a common parameter of the diet. Based on the dairy NRC (2001) data the NDIP might be calculated in the present diets. Although, it was not the aim of this study, a strong negative correlation, $r = -0.82$ was obtained between the percent of NDIP in crude protein of the diets and fecal protein digestibility. Schulze et al. (1994) also mentioned that increased ileal N excretion could be derived from the increased NDF bound N presents in the diet. Further examination is needed to work out how NDIP may be used for predicting protein digestibility in hog diets containing high amount of fiber rich by-products.

Variance component analysis of digestibility of crude protein showed that the fat content of diets contributed a mere 0.9 %, and the impact of fiber was 53.3 % (Table 15).
Table 15. The contribution of fat, fiber and fat x fiber interaction in the total variance (%)

<table>
<thead>
<tr>
<th>Component of variance</th>
<th>Crude protein</th>
<th>Crude fat</th>
<th>Crude fiber</th>
<th>Gross energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat-effect</td>
<td>0.9</td>
<td>51.6</td>
<td>13.2</td>
<td>3.0</td>
</tr>
<tr>
<td>Fiber-effect</td>
<td>53.3</td>
<td>5.0</td>
<td>30.9</td>
<td>86.7</td>
</tr>
<tr>
<td>Fat * fiber interaction</td>
<td>6.7</td>
<td>36.2</td>
<td>35.0</td>
<td>5.2</td>
</tr>
<tr>
<td>Other effects</td>
<td>39.1</td>
<td>7.2</td>
<td>20.9</td>
<td>5.1</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

The impact of interaction between fat and fiber was limited (6.7 %) that refers to relative consistent changes in protein digestibility to increased wheat bran and fat inclusion. Up to about 39.1 %, however, digestibility was influenced by other factors, which can not be explained with the treatment effects. Among these factors the followings might be listed: the fermentation process in the ceacum-colon tract, or the possible catabolism taking place in the colon (Bakker, 1996; Högberg and Lindberg, 2004, Wilfart et al., 2007).

It can be concluded that increasing fiber content clearly decreased the protein digestibility, however, it was also influenced by other factors independent from dietary treatments.

6.1.2 Digestibility of crude fat

The effect of different levels of fat supplementation and wheat bran inclusion on the digestibility of crude fat is shown in Table 16. Increasing fat content of the diet by additional fat significantly increased the fat digestibility in all wheat bran level except from diets contained 600 g/kg wheat bran.
Table 16. The effect of wheat bran (WB) and fat inclusion on total tract digestibility of crude fat (%)

<table>
<thead>
<tr>
<th>WB (%)</th>
<th>0.0</th>
<th>2.5</th>
<th>5.0</th>
<th>7.5</th>
<th>10.0</th>
<th>RMSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>18.6&lt;sup&gt;Cc&lt;/sup&gt;</td>
<td>58.8&lt;sup&gt;Bb&lt;/sup&gt;</td>
<td>64.9&lt;sup&gt;ABab&lt;/sup&gt;</td>
<td>67.5&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>92.7&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>4.5</td>
</tr>
<tr>
<td>15</td>
<td>28.5&lt;sup&gt;Bd&lt;/sup&gt;</td>
<td>55.8&lt;sup&gt;Bc&lt;/sup&gt;</td>
<td>61.8&lt;sup&gt;ABbc&lt;/sup&gt;</td>
<td>66.1&lt;sup&gt;Aab&lt;/sup&gt;</td>
<td>87.4&lt;sup&gt;Bc&lt;/sup&gt;</td>
<td>3.9</td>
</tr>
<tr>
<td>30</td>
<td>34.3&lt;sup&gt;Cc&lt;/sup&gt;</td>
<td>53.6&lt;sup&gt;Bb&lt;/sup&gt;</td>
<td>57.9&lt;sup&gt;Bb&lt;/sup&gt;</td>
<td>67.7&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>83.8&lt;sup&gt;Ch&lt;/sup&gt;</td>
<td>3.5</td>
</tr>
<tr>
<td>45</td>
<td>44.7&lt;sup&gt;Bd&lt;/sup&gt;</td>
<td>51.6&lt;sup&gt;Bc&lt;/sup&gt;</td>
<td>60.8&lt;sup&gt;Bb&lt;/sup&gt;</td>
<td>61.5&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>82.3&lt;sup&gt;Cab&lt;/sup&gt;</td>
<td>3.3</td>
</tr>
<tr>
<td>60</td>
<td>70.9&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>67.9&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>69.9&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>62.8&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>78.6&lt;sup&gt;Da&lt;/sup&gt;</td>
<td>4.6</td>
</tr>
<tr>
<td>RMSE</td>
<td>2.3</td>
<td>4.3</td>
<td>4.4</td>
<td>4.4</td>
<td>4.2</td>
<td>4.1</td>
</tr>
</tbody>
</table>

<sup>ABCD</sup> Within a column, means without a common superscript letter differ (P≤0.05)

<sup>abcde</sup> Within a row, means without a common superscript letter differ (P≤0.05)

RMSE Root mean square error

Diets formulated with no additional fat contained 18-31 g/kg crude fat derived mainly from soy bean meal and wheat. The very low fat digestibility in diet with no additional fat and no inclusion of wheat bran (18.6 %) was unexpected. This was probably caused by relatively high proportion of endogenous fat. Increasing fat digestibility with additional oil was in agreement with our expectations because dietary fat used contained high amount of unsaturated fatty acid (64 % of total fatty acid was unsaturated) which can be absorbed with high efficiency in the small intestine. For diets containing 600 g/kg wheat bran, irrespectively of fat supplementation, high digestibility coefficients were obtained in range from 62.8 to 70.9 %. This can probably be explained by an enhanced pancreatic secretion by generate an increase in the volume and protein output of the pancreatic juice secreted (Langlois et al., 1987; Ikegami et al., 1990; Freire et al., 1998) and lipase activity (Dukehart et al., 1989) promoted by the extra high level of wheat bran.

Changes in digestibility of crude fat as a function of dietary fiber within each fat level were diverse (Table 16). For diets with no added fat,
wheat bran inclusion increased the fat digestibility from 18.7 to 70.9 \%.

There are limited data showing that increasing fiber content of the diet enhances the fat digestibility (Freire et al, 1998; Högberg and Lindberg, 2004), many studies reported *vica versa* (Noblet and Perez, 1993; Bakker et al., 1995; Mroz et al, 1996; Le Goff and Noblet, 2001, Wilfart et al., 2007). The reason for results of this study is probably, that increasing dietary wheat bran increased pancreatic secretion (Isakson et al. 1983; Langlois et al., 1987; Ikegami et al., 1990; Freire et al., 1998), and through that also the activity of lipase secreted by the pancreas increased. The results of this study suggest that increasing fiber content does not increase the fecal fat digestibility when the diet contains fat supplementation. In case of 25 g/kg added fat, the dietary wheat bran content up to 450 g/kg did not change the digestibility of fat being 54.9 \% on average, however, it was 67.9 \% for the diet with 600 g/kg wheat bran. No consequent or significant effect of wheat bran level was found when diets supplemented with 50, 75 or 100 g/kg fat. In agreement with the results of this study, Bach-Knudsen and Hansen (1991) reported no significant effect of inclusion of wheat by-products (30-54 g/kg NSP) on fecal digestibility of fat. Although fiber rich diets may enhance the protein synthesis in the small intestine and the connecting glands such as pancreas (Isakson et al. 1983), probably supplementary fat reduces the abrasive effect of fiber and thus moderates the protein turnover in the viscera (see results of exp. 2). Accordingly at higher fat inclusion (above 50 g/kg) the fat digestibility was not influenced by wheat bran content of the feed.

Analyzing the variance components of fat digestibility showed that the fat content of diets contributed to it by 51.6 \%, and fiber content contributed by 5.0 \% (Table 15). According to the digestibility data it can be seen that the magnitude of the effect of additional fat was much bigger than that of
fiber. The impact of interaction between fat and fiber was also significant, which in this trial proved to be 36.2 %, however the variance of fat digestibility was influenced by other factors up to 7.2 %. This considerable impact of interaction was due to the inconsequent changes of digestibility coefficient at different fat supplementation (Bakker et al., 1995; Jackson et al., 1996). In agreement with results of this study O’Doherty et al. (2002) found that inclusion of fat (25 vs. 50 g/kg) increased the fat digestibility at 50 and 60 g/kg, however, not at 70 g/kg crude fiber level of the diets.

In conclusion, digestibility of crude fat increased consistently by fat supplementation and by inclusion of wheat bran in case of no added dietary fat. When diets were formulated with additional fat, the wheat bran inclusion did not change the digestibility of fat consistently. The effect of fiber is modified by fat content of the diet; therefore due to the fiber x fat interaction the digestible fat content is not predictable accurately by addition of digestible fat content of feed ingredients.

6.1.3 Digestibility of crude fiber

The effect of wheat bran inclusion and fat supplementation on total tract digestibility of crude fiber is shown in Table 17. According to data in this study the additional fat level had no consistent impact on digestibility of fiber.

However, some rat studies reported that high content of dietary fat reduces the fermentation in the hind gut and thus results in lower fiber digestibility (Mallett and Rowland, 1983). In the present study the crude fiber digestibility ranged from 28.5 to 47.2 %. As it was shown above the
Table 17. The effect of wheat bran (WB) and fat inclusion on total tract digestibility of crude fiber (%)

<table>
<thead>
<tr>
<th>WB (%)</th>
<th>0.0</th>
<th>2.5</th>
<th>5.0</th>
<th>7.5</th>
<th>10.0</th>
<th>RMSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Aab</td>
<td>ABc</td>
<td>Aabc</td>
<td>Ac</td>
<td>Aa</td>
<td>3.3</td>
</tr>
<tr>
<td>15</td>
<td>BCbc</td>
<td>DCC</td>
<td>Aab</td>
<td>Abc</td>
<td>Ab</td>
<td>3.0</td>
</tr>
<tr>
<td>30</td>
<td>Bb</td>
<td>BCbc</td>
<td>Aa</td>
<td>Ab</td>
<td>Aa</td>
<td>2.8</td>
</tr>
<tr>
<td>45</td>
<td>CCc</td>
<td>De</td>
<td>Ab</td>
<td>Ab</td>
<td>Aa</td>
<td>2.2</td>
</tr>
<tr>
<td>60</td>
<td>BCc</td>
<td>ACc</td>
<td>Aab</td>
<td>Abc</td>
<td>Aa</td>
<td>2.4</td>
</tr>
<tr>
<td>RMS</td>
<td>2.7</td>
<td>2.4</td>
<td>3.3</td>
<td>2.8</td>
<td>2.6</td>
<td>2.8</td>
</tr>
</tbody>
</table>

*ABCD* Within a column, means without a common superscript letter differ (P≤0.05)

*abcd* Within a row, means without a common superscript letter differ (P≤0.05)

RMSE Root mean square error

additional fat increased the fat digestibility, which acts mainly in the small intestine, and therefore it is likely that the digesta entering the hind gut did not contain enough fat to dramatically reduce the microbial fermentation. On the other hand Bakker (1996) reported that in case of cellulose – which is a major component of wheat bran - the additional fat (60 g/kg) did not change the volatile fatty acid production.

The effect of wheat bran inclusion on fecal digestibility of crude fiber can be described by a minimum curve except from treatments with 75 g/kg added fat level, in which no significant difference was obtained among the digestibility data. In other treatments at each fat level the crude fiber digestibility decreased with increasing wheat bran content of the diets, but at 600 g/kg wheat bran inclusion the fiber digestibility increased consistently compared to the minimum value (P<0.05). When diets contained 25, 50 or 100 g/kg fat and 600 g/kg wheat bran, the digestibility of fiber even reached
the value obtained in the diets without wheat bran. According to the experimental design the major fiber source was from wheat bran that contains about 30 g/kg soluble and 270 g/kg insoluble fiber (Bach-Knudsen, 1997). Different studies show that insoluble fiber is less digestible in the hindgut than soluble fiber (Bach-Knudsen and Jorgensen, 2001; Wang et al, 2002; Höberg and Lindberg, 2004), and according to Stephen and Chummings (1980) and Donangelo and Eggum (1985) wheat bran is considered as a relatively resistant feed ingredient to bacterial fermentation. Therefore it is likely that increasing dietary fiber from wheat bran decreased the fermentation processes in the hindgut and resulted in less digestibility of fiber. However, at extremely high level of dietary fiber (600 g/kg wheat bran) the fiber digestibility was similar than in low fiber diets (contained no wheat bran), which is in agreement with studies of Höberg and Lindberg (2004) and Wilfart et al. (2007).

It can be seen in Table 15 that the variance components within the total variance distributed flatten in case of crude fiber than in other nutrients. Approximately 13 % of the total variance of fiber digestibility is explained by fat supplementation. The fiber content of diets contributes to the total variance by 30.9 %. The interaction between fat and fiber made the stronger impact (35.0 %), but other factors had also relatively high influence (20.9 %). Among undefined factors the physico-chemical property of the fiber and the fermentation capacity of the hindgut are probably the most determinants (Bach-Knudsen and Hansen, 1991; Bakker, 1996; Bach-Knudsen, 2001).

The result of this study suggests that the digestibility of fiber cannot be determined by any well defined factors, however it seems that the fiber level, and the fat x fiber interaction has a relative strong impact on it.
6.1.4 Digestibility of energy

Currently used calculated energy evaluation systems in pig feeding, such as DE, ME and even NE, are based on digestible nutrients present in the diet. However, in many cases addition of digestible nutrient content of the feed components fails to give the accurate digestible nutrient and energy content of the mixed feed even in fiber rich diets. The effect of different levels of dietary fiber has been investigated by many studies. It has been frequently cited that dietary fibers influence the digestive processes and results in reduced rate of nutrient absorption. Some studies reported that due to the fiber x fat interaction prediction of digestible energy content of the feed is even less precise for diets containing high fiber and high fat. Present trial was conducted to study the effect of fiber and fat and their interaction on the nutrient and in particular energy digestibility at the total tract level.

The digestibility of the energy content of the diets is shown in Table 18. According to the results of this study the fat content has slight impact on the digestibility of gross energy, with increasing fat content it decreases in most cases to a small extent (P<0.05). This was unexpected, because additional fat increased the fat digestibility and obviously the gross energy of the diet, did not influence the protein digestibility and did not reduce consistently the fiber digestibility. It seems fiber has a stronger effect on the digestibility of gross energy. Therefore, it might be caused by a reduced digestibility of N-free extract. However, no literature was found reporting that increasing fat supplementation would decrease the absorption rate of N-free extract.
Table 18. The effect of wheat bran (WB) and fat inclusion on total tract digestibility of gross energy (%)

<table>
<thead>
<tr>
<th>WB (%)</th>
<th>0.0</th>
<th>2.5</th>
<th>5.0</th>
<th>7.5</th>
<th>10.0</th>
<th>RMSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>87.7^Aa</td>
<td>86.1^{Aab}</td>
<td>85.5^{Abc}</td>
<td>83.7^{Ac}</td>
<td>83.9^{Ac}</td>
<td>1.0</td>
</tr>
<tr>
<td>15</td>
<td>82.3^{Ba}</td>
<td>82.4^{Ba}</td>
<td>81.2^{Bab}</td>
<td>80.6^{Bb}</td>
<td>80.1^{Bb}</td>
<td>0.9</td>
</tr>
<tr>
<td>30</td>
<td>80.3^{Ca}</td>
<td>79.0^{Ca}</td>
<td>80.7^{Bab}</td>
<td>78.7^{Bab}</td>
<td>76.3^{Cb}</td>
<td>1.4</td>
</tr>
<tr>
<td>45</td>
<td>76.3^{Da}</td>
<td>73.7^{Dab}</td>
<td>72.8^{Chc}</td>
<td>73.4^{Cc}</td>
<td>76.9^{Ca}</td>
<td>1.4</td>
</tr>
<tr>
<td>60</td>
<td>74.8^{Da}</td>
<td>73.7^{Da}</td>
<td>74.8^{Ca}</td>
<td>71.1^{Db}</td>
<td>73.2^{Da}</td>
<td>1.2</td>
</tr>
<tr>
<td>RMSE</td>
<td>1.0</td>
<td>1.0</td>
<td>1.4</td>
<td>1.1</td>
<td>1.4</td>
<td>1.2</td>
</tr>
</tbody>
</table>

^A^B^C^D^E^ Within a column, means without a common superscript letter differ (P≤0.05)

^a^b^c^d^e^ Within a row, means without a common superscript letter differ (P≤0.05)

RMSE Root mean square error

With increasing fiber content the digestibility of energy decreased significantly (P<0.05) which is in agreement with several studies (Noblet and Perez, 1993; Le Goff and Noblet, 2001). In general, digestibility of starch - the major component of N-free extract - is almost complete and it is hardly affected by different types of polysaccharides presented in the pig feed (Drochner et al., 1984; Bach-Knudsen and Hansen, 1991; Bakker, 1996). Detrimental effect of dietary fiber on energy digestibility is likely cumulated from reduced digestibility of protein, fiber and fat.

As it can be seen from the data of variance component analysis (Table 15), the changes in energy digestibility are primarily caused by the crude fiber content of the diets (86.7 %). Fat content of the diets contribute to the total variance by 3.0 % only, whereas the interaction between fat and fiber as well as other non defined factors contribute similar magnitude by 5.2 and 5.1 %, respectively. The very strong effect of fiber is probably due to the fiber source. Wheat bran contains mainly insoluble fiber that is hardly fermented by the bacteria and it alters the digestibility of other nutrients.
The results also show that the effect of fiber x fat interaction on digestibility of energy is negligible and the digestible energy content is well predictable from the gross energy and the fiber content of the diet. Bakker et al. (1995) found a significant fat x fiber interaction on the digestible energy content of the feed. They used animal fat with high content of saturated fatty acid, however they discussed that the fat type could be the determinant in existence of this interaction.

6.2 The effect of wheat bran and soyhulls inclusion on the standardized ileal digestibility of selected amino acids

6.2.1 Ileal excretion of endogenous amino acids

In the pretrial ileal excretion of endogenous amino acids was determined in order to calculate the standardized ileal digestibility of the wheat bran and soy hulls trial. However, it was not the aim of the study to investigate the endogenous fraction in detail. Changes of the ileal excretion of endogenous amino acids are presented in Table 19. According to data in this study the average N excretion of the experimental animals was 2605 mg/kg DM intake, and there was no statistically verifiable difference among dietary treatments \( P \geq 0.05 \). It was remarkable that the increased fiber content of the diet did not result in increased ileal excretion of endogenous N and amino acids, moreover we found decreased N and amino acid loss.
Table 19. Ileal-endogenous excretion of amino acids (mg/kg DM intake)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TREATMENTS</th>
<th>RMSE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td><strong>Amino acids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrogen</td>
<td>2722&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2645&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lysine</td>
<td>626&lt;sup&gt;a&lt;/sup&gt;</td>
<td>596&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Methionine</td>
<td>93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cystine</td>
<td>193&lt;sup&gt;a&lt;/sup&gt;</td>
<td>158&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Methionine+Cystine</td>
<td>286&lt;sup&gt;a&lt;/sup&gt;</td>
<td>215&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Threonine</td>
<td>721&lt;sup&gt;a&lt;/sup&gt;</td>
<td>695&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>204&lt;sup&gt;a&lt;/sup&gt;</td>
<td>207&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Arginine</td>
<td>1236&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1182&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>464&lt;sup&gt;a&lt;/sup&gt;</td>
<td>445&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Valine</td>
<td>647&lt;sup&gt;a&lt;/sup&gt;</td>
<td>603&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

**Abc** Within a row, means without a common superscript letter differ (P≤0.05)

RMSE Root mean square error

A ; B ; C ; D treatments: the isocaloric N free diets, see Table 6.

Many studies showed that the addition of cellulose to the diet has no effect on ileal endogenous N excretion (de Lange et al., 1989; Furuja and Kaji, 1992; Leterme et al., 1992, Babinszky et al., 2007), however purified NDF or a natural source of NDF from wheat bran induced it (Schultze et al., 1994; Yin et al., 2000).

In general available literature data show (Furuya and Kaji, 1992; Schultze et al., 1994; Yin et al., 2000) that increasing amount of fiber in the diets results increased ileal excretion of endogenous N and amino acids. The reason of the slightly decreasing endogenous amino acid loss in this trial is probably due to the isocaloric feeds. In order to achieve similar energy
concentration in all diets, parallel to the increase of crude fiber content the feeds were supplemented with fat.

In agreement with this Li and Sauer (1994) found that increasing dietary fat level increased the ileal amino acid digestibility. This draws attention to the need to consider the fat content of the diets while determining the excretion of endogenous amino acids.

6.2.2 The effect of wheat bran inclusion on the standardized ileal digestibility of selected amino acids

The standardized ileal digestibility of amino acids in diets with different wheat bran content is presented in Table 20. According to data in this study the SID of lysine was not influenced by the wheat bran level (P>0.05). The average SID of methionine, cystine and methionine+cystine was 2.4, 3.1 and 2.8 % lower, respectively in diets with wheat bran supplementation (WB-25, WB-50, WB-75, WB-100) compared to the control (WB-0). Significant differences (P≤0.05) were obtained between diets WB-0 and WB-75 in the case of methionine and between diets WB-0 and WB-50 in the case of cystine. In diets containing 50, 75 and 100 g/kg wheat bran, the digestibility of Met+Cys was lower than in the control diet (P≤0.05). Increasing the level of wheat bran decreased the digestibility of threonine and tryptophan, however, a significant difference was only obtained between treatments WB-0 and WB-100.
Table 20. The effect of wheat bran inclusion on the standardized ileal digestibility of selected amino acids (%)

<table>
<thead>
<tr>
<th></th>
<th>WB-0</th>
<th>WB-25</th>
<th>WB-50</th>
<th>WB-75</th>
<th>WB-100</th>
<th>RMSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>84.7a</td>
<td>83.9a</td>
<td>83.0a</td>
<td>83.3a</td>
<td>83.1a</td>
<td>2.2</td>
</tr>
<tr>
<td>Methionine</td>
<td>87.2a</td>
<td>85.1ab</td>
<td>84.7ab</td>
<td>84.6b</td>
<td>84.8ab</td>
<td>1.8</td>
</tr>
<tr>
<td>Cystine</td>
<td>80.4a</td>
<td>77.7ab</td>
<td>76.9b</td>
<td>77.2ab</td>
<td>77.4ab</td>
<td>2.4</td>
</tr>
<tr>
<td>Met+Cys</td>
<td>83.9a</td>
<td>81.6ab</td>
<td>80.8b</td>
<td>80.9b</td>
<td>81.2b</td>
<td>1.7</td>
</tr>
<tr>
<td>Threonine</td>
<td>77.5a</td>
<td>76.3ab</td>
<td>74.5ab</td>
<td>73.9ab</td>
<td>73.2b</td>
<td>2.8</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>86.8a</td>
<td>85.8ab</td>
<td>85.7ab</td>
<td>84.1ab</td>
<td>83.5b</td>
<td>1.9</td>
</tr>
</tbody>
</table>

Within a row, means without a common superscript letter differ (P≤0.05)

RMSE Root mean square error

The relationship between dietary wheat bran level and SID of amino acids was analyzed when a significant difference was obtained among dietary treatments (Table 21). In the case of methionine and cystine the best fit was found with linear-plateau curve. To gain a better understanding the linear-plateau function with sharp transition ([Y = a0 − a1* ln(1 + exp(a2 − X))]) was chosen in which a0 is the value of the plateau on the Y axis, a1 is the slope of the linear phase and a2 is the transition point between the two phases on the X axis (Figure 7).
Table 21. Relationship between wheat bran or soyhulls level of the diet (X, g/kg) and the standardized ileal digestibility of amino acids (Y, %)

<table>
<thead>
<tr>
<th></th>
<th>Y = a₀ + a₁X</th>
<th>a₀</th>
<th>a₁</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB</td>
<td>Thr</td>
<td>77.3</td>
<td>-0.044</td>
<td>0.004</td>
</tr>
<tr>
<td>WB</td>
<td>Trp</td>
<td>86.8</td>
<td>-0.033</td>
<td>0.005</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Y = a₀ + a₁X + a₂X²</th>
<th>a₀</th>
<th>a₁</th>
<th>a₂</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SH</td>
<td>Thr</td>
<td>77.2</td>
<td>-0.221</td>
<td>0.0015</td>
<td>0.043</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Y = a₀ - a₁* ln(1 + exp(a₂ - X))</th>
<th>a₀</th>
<th>a₁</th>
<th>a₂</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB</td>
<td>Met</td>
<td>84.7</td>
<td>-0.084</td>
<td>29.8</td>
<td>0.004</td>
</tr>
<tr>
<td>WB</td>
<td>Cys</td>
<td>77.2</td>
<td>-0.108</td>
<td>29.9</td>
<td>0.016</td>
</tr>
<tr>
<td>WB</td>
<td>Thr</td>
<td>73.5</td>
<td>-0.060</td>
<td>67.5</td>
<td>0.024</td>
</tr>
<tr>
<td>SH</td>
<td>Lys</td>
<td>77.8</td>
<td>-0.201</td>
<td>31.0</td>
<td>0.001</td>
</tr>
<tr>
<td>SH</td>
<td>Met</td>
<td>82.4</td>
<td>-0.154</td>
<td>29.0</td>
<td>0.013</td>
</tr>
<tr>
<td>SH</td>
<td>Cys</td>
<td>73.7</td>
<td>-0.171</td>
<td>37.2</td>
<td>0.001</td>
</tr>
</tbody>
</table>

WB: wheat bran, SH: soyhulls

\[ Y = a_0 - a_1 \ln(1 + \exp(a_2 - X)) \]

Figure 7. Linear-plateau function with sharp transition
The effects of fiber or NDF level on the digestibility of protein and amino acids are usually described by linear regression (Sauer et al., 1991; Schultze et al., 1994; Lenis et al., 1996; Yin et al., 2000). This is the first time that the linear-plateau function was used to characterize the relationship between the level of fiber sources and SID of amino acids. The linear-plateau method is a two-phase-figure with a slope and a constant phase, and shows that after a certain level of wheat bran or soyhulls inclusion, the digestibility of amino acids do not decline further. According to the results of Yin et al. (2000) the decreasing effect of fiber on apparent digestibility of amino acids was mainly the result of higher endogenous amino acid loss. If this is the case also in the present study, then the linear-plateau function demonstrates that the EAAL increases up to a certain fiber level and then remains constant. This hypothesis is supported by Taverner et al. (1981) who suggested that the ileal output of endogenous N increased with dietary fiber up to approximately 100 g NDF/kg, but not thereafter. Taverner et al. (1981) used wheat and cellulose for increasing NDF content of the diet. The data in this study shows, that incremental inclusion of wheat bran beyond 30 g/kg up to 100 g/kg did not change the SID of methionine and cystine. The relationship between wheat bran inclusion and SID of threonine can be described both by linear regression (P=0.004) and linear-plateau figure (P=0.024). SID of tryptophan decreased linearly with wheat bran inclusion (P<0.05).
6.2.3 The effect of soyhulls inclusion on the standardized ileal digestibility of selected amino acids

The effect of soyhulls level of the diet on the standardized ileal digestibility of amino acids is shown in Table 22.

Table 22. The effect of soyhulls inclusion on the standardized ileal digestibility of selected amino acids (%)

<table>
<thead>
<tr>
<th></th>
<th>SH-0</th>
<th>SH-25</th>
<th>SH-50</th>
<th>SH-75</th>
<th>SH-100</th>
<th>RMSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>84.2</td>
<td>79.0</td>
<td>78.0</td>
<td>77.8</td>
<td>77.8</td>
<td>2.4</td>
</tr>
<tr>
<td>Methionine</td>
<td>86.9</td>
<td>83.1</td>
<td>82.7</td>
<td>83.0</td>
<td>82.1</td>
<td>1.9</td>
</tr>
<tr>
<td>Cystine</td>
<td>80.0</td>
<td>75.7</td>
<td>73.8</td>
<td>73.5</td>
<td>73.6</td>
<td>1.7</td>
</tr>
<tr>
<td>Met+Cys</td>
<td>83.6</td>
<td>79.5</td>
<td>78.4</td>
<td>78.2</td>
<td>78.1</td>
<td>1.6</td>
</tr>
<tr>
<td>Threonine</td>
<td>76.9</td>
<td>73.4</td>
<td>68.9</td>
<td>69.7</td>
<td>70.1</td>
<td>2.4</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>86.5</td>
<td>86.6</td>
<td>82.2</td>
<td>82.4</td>
<td>82.8</td>
<td>2.5</td>
</tr>
</tbody>
</table>

a, b Within a row, means without a common superscript letter differ (P≤0.05)
RMSE Root mean square error

Inclusion of SBH depressed the digestibility of all investigated amino acids. In the cases of lysine, methionine and cystine, the SID values were significantly lower (with 5.9, 4.3 and 5.8 % respectively) than in the control when soyhulls were not included in the diets.

However, the level of soyhulls had no influence on digestibility of these amino acids. Increasing the level of soyhulls up to 50 g/kg (SH-50) reduced the digestibility of threonine and tryptophan from 77 to 69 % and from 87 to 82 %, respectively (P≤0.05). Higher inclusion rates of fiber did not depress the digestibility of threonine and tryptophan. It has to be
acknowledged, however, that the protein quality of soyhulls has been reported to be equivalent to soybean meal (Kognegay, 1978) and Dilger et al. (2004) suggested that main effect of soyhulls on digestion arises largely from its high dietary fiber content. In the present study beyond 25 and 50 g/kg soyhulls inclusion the digestibility of amino acids was not influenced significantly by treatments. In accordance with the results of this study Dilger et al. (2004) also found that inclusion of 30 and 60 g/kg soyhulls decreased the digestibility of amino acids, but 90 g/kg soyhulls (65.6 g/kg dietary NDF) did not reduce further the standardized ileal digestibility of lysine, methionine, cystine, threonine and tryptophan as well as some other amino acids compared to diets containing 60g/kg soyhulls (75.9 g/kg dietary NDF). It is likely from the discussion above, that the source of NDF is also a determining factor in regards to the transition point of the linear-plateau function.

Results of the regression analysis are presented in Table 21. The relationship between dietary soyhulls level and SID of lysine, methionine and cystine can be accurately described with the linear plateau function. The equation of linear plateau function with sharp transition shows that increasing the level of soyhulls between 30 and 100 g/kg did not affect the lysine and methionine digestibility and above 37 g/kg inclusion of soyhulls the cystine digestibility did not change. In the case of threonine the quadratic regression was significant (P<0.05). The quadratic equation shows that SID of threonine has a minimum value at 74 g/kg of soyhulls inclusion. In the case of tryptophan neither linear, quadratic nor the linear-plateau curve could be fitted to the plots (P>0.05).
6.2.4. The comparison of the effect of different levels of dietary wheat bran and soyhulls on the standardized ileal digestibility of amino acids

In agreement with several studies, data in this study shows that increasing the level of dietary NDF either from wheat bran or soyhulls decreases the ileal amino acid digestibility (Bach-Knudsen and Hansen, 1991; Lenis et al., 1996; Huang et al., 1999; Yin et al., 2000; Dilger et al., 2004). The reason can be explained by different factors. First, some protein and amino acids are present in the NDF matrix and those are unavailable during the small intestinal digestion (Schulze, 1994). According to NRC (2001) both wheat bran and soyhulls contain a significant amount of NDF bounded N (25% and 16% of total protein, respectively). Furthermore, the reducing effect of NDF on the SID of amino acids found in this study can probably be derived from the larger specific endogenous amino acid losses. SID is used by correcting apparent ileal amino acid digestibility for basal endogenous amino acid contributions and ignoring the specific endogenous N. It has been reported that the amount of total endogenous N, originated from enzymes, mucus, and protein from desquamated cells, is linearly related to the amount of consumed NDF (Furuya and Kaji, 1992; Schulze, 1994) likely due to the increased specific endogenous nitrogen loss (ENL) and endogenous amino acid loss (EAAL). In a recent study, Yin et al. (2000) concluded that the depression in apparent ileal digestibility of protein with increased dietary NSP is mainly due to increased ileal endogenous N. Furthermore, in cases of wheat bran and soyhulls inclusion, the increasing level of NDF bounded protein which is indigestible, might result in a higher proteolytic activity leading to an accelerated erosion of the mucus layer (Piel et al., 2007) and/or it acts as a factor stimulating mucus secretion.
(Santoro et al., 1999) and reducing its reabsorption (Mosenthin et al., 1994; Grala et al., 1998). Thus a higher endogenous amino acid level present in the digesta can be one of the reasons of the depressive effect of NDF on SID of amino acids in our study. It is generally observed that the feed intake and dry matter intake highly correlate with the EAAL (Butts et al., 1993). In this study the inclusion of fiber sources resulted in decreasing the ME content and in order to maintain the same energy supply (2.6 times of maintenance energy requirement) the daily feed intake of the pigs increased with increasing wheat bran and soyhulls level of the diets. The higher daily proportion of feed (and dry matter) with increasing levels of NDF source might also cause somewhat higher endogenous N loss and thus resulted in a reduced standardized ileal digestibility of amino acids in the present study.

It can be concluded from the regression analysis that the magnitude of the effect of soyhulls on the digestibility data was larger than that of wheat bran. The slope of the regression was between -0.15 and -0.20 for soyhulls and ranged from -0.03 to -0.11 for wheat bran diets. The quantitative increase in fiber fractions supplied by soyhulls may have induced greater losses of amino acid containing sources such as digestive enzymes, enterocytes and mucus (Dilger et al., 2004). The reason for it is complex and probably includes type and solubility of fiber and presence of antinutritional factors. Soyhulls contain undigestible oligosaccharides like xylan (Aspinall et al., 1966) that may act as antinutritive factor (ANF). Hemicellulose and other oligosaccharides in wheat bran and soy hulls act as ANF. The variable ANF contents of feedstuffs are known to affect intestinal mucus secretion and may affect EAAL (Myrie et al., 2008). It has been frequently reported that the solubility of fiber might influence the endogenous secretion of protein. Feed that contains soluble fiber increases the water-holding capacity and thus the viscosity of the digesta, causing an increase in physical
abrasion of epithelial cells by the digesta (Sauer, 1976). Considering that soyhulls and wheat bran contain 830 and 450 g/kg total dietary fiber with an insoluble to soluble fiber ratio of 5.0 (Dust et al., 2004) and 14.0 (Bach-Knudsen, 1997), respectively it is understandable that soyhulls inclusion reduces more the SID of amino acids than wheat bran.

The bigger depressive effect of soyhulls inclusion on the digestibility of amino acids can be explained, at least partly, by a higher bacterial mass presented in the small intestine (Schutte et al., 1991; Yin et al., 2000). The soluble fiber fraction of the diet might increase the bacterial mass in the ileum (Lien et al., 1997) and thus reduces the digestibility of N containing compounds. However, the total small intestinal fermentation is limited and is much less than hindgut microbial fermentation. About 1 % of the dry matter was fermented in the ileum in study of Yin et al. (2000) when half of wheat (380g/kg) was substituted by wheat bran or wheat middlings. Wheat bran is considered as a relatively resistant compound to bacterial fermentation (Stephen and Chummings, 1980; Donangelo and Eggum, 1985), therefore the influence of microbial mass is probably negligible in case of wheat bran inclusion.

6.3 General discussion

The trials carried out showed that fiber clearly has a depressing effect on fecal digestibility of protein and energy and also decreases the ileal digestibility of amino acids. These effects can be computed into diet formulation.

The simplistic use of only book values may alter the accuracy of the desired diet. Diet formulation should be based on laboratory analysis and key nutrients should be updated continuously. However further nutrient interactions also can influence the precise diet formulation. Based trials
completed in this study some model calculation can be introduced in order to enhance the accuracy of diet formulation

6.3.1 Consequences of fiber x fat interaction on diet formulation

Determination of DE content of the feed is crucial in diet formulation. For that purpose Schieman equation for swine (Schieman et al. 1972) are widely used, however, the limitation of the formula’s application is that implies digestible nutrient content. Moreover, DE content of the feed can be also calculated by multiplying the gross energy content of the feed by its digestibility coefficient. In that case the coefficient has to be determined directly or indirectly by regression equations. Whenever the DE content of the feed was calculated by regressions an accurate prediction of digestibility coefficients for nutrients and energy would be required. Many studies have published regressions to predict the digestibility of nutrients or the digestible nutrient content of the feed as a function of dietary nutrients, particularly fiber, NDF or ash (Noblet and Perez, 1993; Le Goff and Noblet, 2001; Drochner et al., 2004). However, it has been also found that in case of high fiber and high fat diet the interaction between fiber and fat can modify the digestibility coefficient of nutrients and energy.

The results of the multivariate linear regression presented in Table 23 show that the digestibility coefficients of crude protein, ether extract and crude fiber can be predicted with low accuracy ($R^2 < 0.50$). This is a logical consequence of results of variance component analysis, considering that in case of protein digestibility the undefined effect or in cases of fat and fiber digestibility the effect of fiber x fat interaction was significant. Due to the fact that the impact of fat and fiber contributed approximately 90 % of the total variance of the data, the accuracy of the regression that computes the energy digestibility is quite high ($R^2 = 0.845$).
Table 23. Relationships between crude fat (EE), crude fiber (CF) and N-free extract (NFE) contents of the feed (g/kg) and nutrient and energy digestibility (%) using multiple linear regression

<table>
<thead>
<tr>
<th>Nutrient Digestibility</th>
<th>RMSE</th>
<th>$r^2$</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein digestibility</td>
<td>2.200</td>
<td>0.48</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>intercept</td>
<td>90.296</td>
<td>0.698</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>EE</td>
<td>0.013</td>
<td>0.0062</td>
<td>0.0316</td>
</tr>
<tr>
<td>CF</td>
<td>-0.136</td>
<td>0.0128</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Crude fat digestibility</td>
<td>10.256</td>
<td>0.50</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>intercept</td>
<td>30.457</td>
<td>3.219</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>EE</td>
<td>0.284</td>
<td>0.0286</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CF</td>
<td>0.186</td>
<td>0.0589</td>
<td>0.0019</td>
</tr>
<tr>
<td>Crude fiber digestibility</td>
<td>5.324</td>
<td>0.17</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>intercept</td>
<td>1.417</td>
<td>16.549</td>
<td>0.93</td>
</tr>
<tr>
<td>EE</td>
<td>0.124</td>
<td>0.0340</td>
<td>0.0004</td>
</tr>
<tr>
<td>CF</td>
<td>-0.076</td>
<td>0.0308</td>
<td>0.0155</td>
</tr>
<tr>
<td>NFE</td>
<td>0.055</td>
<td>0.0256</td>
<td>0.0347</td>
</tr>
<tr>
<td>N-free extract digestibility</td>
<td>1.768</td>
<td>0.88</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>intercept</td>
<td>90.995</td>
<td>0.192</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CF</td>
<td>-0.288</td>
<td>0.0102</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>NFE</td>
<td>0.014</td>
<td>0.0037</td>
<td>0.0003</td>
</tr>
<tr>
<td>Energy digestibility</td>
<td>1.875</td>
<td>0.84</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>intercept</td>
<td>92.208</td>
<td>0.589</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>EE</td>
<td>-0.021</td>
<td>0.0052</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CF</td>
<td>-0.264</td>
<td>0.0108</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Based on data in this study a model calculation can be established to see the magnitude of the prediction error of dietary DE content using regression equations computed either from energy or nutrient digestibility.
coefficients. For that purpose two comparisons are done: i) measured vs. simply calculated DE based on digestibility coefficient of energy from regression (see in Table 23), or ii) measured vs. multiple calculated DE. The steps of the multiple calculations are the following: 1) prediction of nutrient digestibility as a function of total nutrient content as shown in Table 23, calculation of DE content of the feed with Schiemann equation based on calculated digestible nutrient content of the feed Schiemann et al. (1972). The equations for nutrients and energy were established by assuming that in the present trial ether extract, crude fiber, and N-free extract content of the feed were the main affecting factors for the digestibility, due to the fact that the amount of them changed in a wide range. The impact of N-free extract on the digestibility coefficient of crude protein, ether extract and energy was not significant (P>0.05). The effect of crude fiber on digestibility of N-free extract was not improved statistically (P>0.05). As an indicator for the error of predicted values relative to the observed values, the mean square prediction error (MSPE) was calculated: \( \text{MSPE} = \frac{\sum (O_i - P_i)^2}{n} \) in which \( O_i \) and \( P_i \) are the observed and predicted values; \( i = 1, \ldots, n \), and \( n = \) number of experimental observations (Bibby and Toutenburg, 1977). The root MSPE is a measure in the same units as the output and it is expressed as a percentage of the observed mean (relMSPE). The MSPE may be decomposed into three fractions. Firstly, errors attributed to overall bias (B %) represent the proportion of MSPE due to a consistent over- or underestimation of the experimental observations by the model predictions. Secondly, deviation of regression slope from one, being the line of perfect agreement (R %) represents the proportion of MSPE due to inadequate prediction of differences between experimental observations. Thirdly, disturbance proportion (E %) represents the proportion of MSPE unrelated to the errors of model prediction. The prediction is very good if the MSPE is small and if
a small proportion of MSPE is explained by the regression error and the deviance in bias.

The comparisons of observed (measured) dietary DE content and predicted DE content of the feed by simple and multiple calculations is presented in Figure 8 and 9, respectively. The data in this study show that the regression equation as a function of ether extract and crude fiber predicts the energy digestibility with a high accuracy. The error of the prediction is 2.4% that are attributed totally to the data disturbance. We expected that multiple calculation predicted the DE content of the feed with a low accuracy considering that the total variance of the data, particularly for fiber and fat digestibility was affected by fiber x fat interaction, and moreover the value of the regression coefficient of protein, fat and fiber was low. However, the results of this study show that the DE content of the feed can also be predicted quite well with multiple calculations (Figure 9). The prediction error is about 0.56 MJ/kg resulting a relMSPE of 4.0%. The overall bias of dietary DE content is predicted precisely (0.5% of MPSE) and the prediction error is mainly due to the data disturbance, attributing 91% of MSPE. The error of the method can be determined by comparing the measured DE to the DE value calculated from Schieman equation that based on measured nutrient digestibility. In this comparison the root MSPE is 0.40 MJ/kg, which is 2.9% of the bias and the error is mainly due to the data disturbance (B %=1.1, R %=3.2, E %=95.7). Therefore, it can be concluded that the multiple linear regression sufficiently predicts the digestibility of energy as a function of dietary fat and fiber within a wide range of dietary fat and wheat bran inclusion. The prediction error is still moderate but increased with 70% (from 0.328 to 0.555 MJ/kg) when the DE was computed with multiple calculation compared to simple calculation.
MSPE = 0.328 MJ/kg, relMPSE = 2.38 %, B % = 0, R % = 0, E % = 100

Figure 8. Comparison of measured dietary DE content and calculated DE content from energy digestibility (regression equation for energy digestibility is presented in Table 23)

MSPE = 0.555 MJ/kg, relMPSE = 4.04 %, B % = 0.5, R % = 8.8, E % = 90.7

Figure 9. Comparison of measured dietary DE content and multiple calculated DE content of the feed using Schieman equation (regression equations for digestibility of nutrients are presented in Table 23)
6.3.2 Consequences of the dietary impact of NDF on diet formulation

The data from the experiment carried out shows that increasing the level of NDF from dietary wheat bran or soyhulls reduced the standardized ileal digestibility of amino acids. The reduction in digestibility coefficients was greater when soyhulls were included to the diet.

Quantification of the depression effect of fiber has significant importance in diet formulation. Based on the computed regressions the standardized ileal digestible amino acid content of the diets formulated with wheat bran and soyhulls inclusion can be calculated. From that data the required amino acid supplementation can be determined.

There is a linear relation between wheat bran level and SID of threonine and tryptophan (Table 21), therefore the required amino acid supplementation for each g of wheat bran inclusion per kg feed (AAsuppl, g/kg) can be calculated with the following equation (eq 1):

\[
\text{AAsuppl} = -a_1 \times \text{SIDAA}(@\text{WB}=0)
\]  
(eq 1)

where \(a_1\) is the slope of the linear regression (taken from Table 21) and \(\text{SIDAA}(@\text{WB}=0)\) is the standardized ileal digestible amino acid content of the diet that contains no wheat bran. The equation 1 shows that each g of wheat bran inclusion up to 1 kg of feed results in 0.002 and 0.001 g/kg reduction in digestible threonine and tryptophan content of the feed, respectively. This has to be compensated for in diet formulation.

The quadratic relation occurring between soyhulls inclusion and SID of threonine demonstrates that there is a minimum value until the SID decreases. This minimum of the curve is computed by the following formula (eq 2):
\[ X_{\text{min}} = -a_1/2a_2 \]  
(eq 2)

where \( a_1 \) and \( a_2 \) are taken from Table 6 and result in \( X_{\text{min}} = 73.7 \) g/kg. The function at this value is (eq 3):

\[ Y(\@X_{\text{min}}) = 0.691 \]  
(eq 3)

The maximum reduction in SID of threonine (\( \text{max} \Delta \text{SID} \)) is (eq 4):

\[ \text{max} \Delta \text{SID} = Y(\@X=0) - Y(\@X_{\text{min}}) = 0.769 - 0.691 = 0.078 \]  
(eq 4)

The maximum requirement of extra threonine supplementation (\( \text{max} \text{Thr} \)) if soyhulls are used in diet formulation is (eq 5):

\[ \text{max} \text{Thr} = \text{max} \Delta \text{SID} \times AA(\@X=0) \]  
(eq 5)

Therefore maximum \( 0.078 \times 6.6 \) g/kg = 0.52 g threonine has to be supplemented when the diet is formulated with soyhulls.

The relationship between wheat bran level and SID of methionine, cystine and threonine as well as between soyhulls level of the diets and SID of lysine, methionine and cystine is well described by the linear-plateau function. The two phase curve shows that after a certain level of wheat bran or soyhulls, the digestibility of amino acids do not decline further and thus no extra supplemental amino acid is required to maintain the digestible amino acid content of the feed. The calculation of the maximum amino acid supplementation can be reviewed in Table 24.
Table 24. Calculation of the additional amino acid (AA) supplementation required after the transition point of the linear-plateau relationship

<table>
<thead>
<tr>
<th></th>
<th>In feed contains no by-product</th>
<th></th>
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</tr>
</thead>
</table>
|                | AA
\((@X=0)\) (g/kg) | SID\(_{AA}\) (% | SIDAA (g/kg) | \(a_1\) (%)  | \(a_2\) (g/kg) | AAsuppl (g/kg) |
| WB Methionine  | 3.1                           | 86.0           | 2.67           | -0.084         | 29.8           | 0.067          |
| WB Cystine     | 2.9                           | 78.9           | 2.29           | -0.108         | 29.9           | 0.074          |
| WB Threonine   | 6.6                           | 75.7           | 4.99           | -0.06          | 67.5           | 0.202          |
| SH Lysine      | 10                            | 81.1           | 8.11           | -0.201         | 31.0           | 0.505          |
| SH Methionine  | 3.1                           | 84.8           | 2.63           | -0.154         | 29.0           | 0.117          |
| SH Cystine     | 2.9                           | 77.1           | 2.24           | -0.171         | 37.2           | 0.142          |

WB: wheat bran, SH: soyhulls, AA
\((@X=0)\): total amino acid content of the diet contained no by-product (from Table 9 and 10), SID\(_{AA}\): standardized ileal digestibility of amino acids calculated from the linear-plateau regression, SIDAA: standardized ileal digestible amino acid content, \(a_1\): slope of the regression of the linear phase of linear-plateau relationship (from Table 21), \(a_2\): transition point of the linear-plateau relationship (from Table 21), AAsuppl: required additional amino acid supplementation.

The standardized amino acid content of the feed (SIDAA
\((@X=0)\)) that contained no by-product is (eq 6):

\[
\text{SIDAA}\(_{(@X=0)}\) = \text{AA}\(_{(@X=0)}\) \times \text{SID}_{AA}
\]

(eq 6)

where AA
\(_{(@X=0)}\) is the total amino acid content of the feed and SID_{AA} is the SID of amino acids calculated from the linear-plateau equations (Table 21).

The required amino acid supplementation (AAsuppl) is (eq 7):

\[
\text{AAsuppl} = \text{SIDAA}\(_{(@X=0)}\) \times a_1 \times a_2
\]

(eq 7)

where \(a_1\) is the slope of the linear phase of the linear-plateau function and \(a_2\) is the transition point of the linear-plateau equation. Based on this equation,
0.067 g methionine, and 0.074 g cystine per each kg of feed is required above 30 g/kg of wheat bran inclusion and 0.202 g threonine per each kg of feed is required above 68 g/kg of wheat bran inclusion. More than 30 g/kg of soyhulls inclusion in the feed results 0.505 and 0.117 g extra lysine and methionine supplementation required per kg feed and more than 37 g/kg of soyhulls results in 0.142 g additional cystine per kg feed required to maintain the standardized amino acid content of the diet.

It can be concluded from the model calculation, that due to the linear-plateau or quadratic relationships between the dietary by-product level and the standardized amino acid digestibility, that in diet formulation the lysine, methionine, cystine should not be increased after 37.2 g/kg soyhulls inclusion, and methionine, cystine after 29.9 g/kg and 67.5 g/kg wheat bran inclusion.

Theoretically if a hog diet is formulated based on adequate amount of ileal digestible amino acids and net energy and other nutrients the animal performance remain the same (Just, 1984). Jongbloed et al., 1986; Bakker, 1996 demonstrated that pigs performed worse when offered diets with a similar calculated net energy supply but composed of by-products plus supplementary fat, compared to pigs given diets based on cereals or by-products without supplemented fat. The present thesis aimed to study the role of fiber in absorption level, however, it is likely that dietary fiber has further impact at metabolic level as well, and therefore it might compromise the animal performance. To compute the effect of fiber on protein, amino acids, and energy digestibility will enable the animal nutritionist to formulate with higher precision. However many open concerns still remain.
7. Conclusions and suggestions
The following conclusions can be drawn from the thesis:

1) Data from the experiment and the results of the variance component analysis show that among the factors of fiber, fat and fiber x fat interaction, the total tract digestibility of protein was mainly determined by the dietary fiber content (53%).

2) Although fiber and fat digestibility were significantly (P≤0.05) influenced by fiber x fat interaction, the digestibility of energy was principally affected by dietary fiber content. The contribution of fiber effect within the total variance of energy digestibility was 87% when wheat bran up to 60% and supplemental fat up to 10% were used in diet formulation.

3) Inclusion of wheat bran and soyhulls depress the standardized ileal digestibility of amino acids with different magnitude. Whilst 2.5% soyhulls inclusion significantly decreases the digestibility coefficients in case of wheat bran, the statistically proven reduction in digestibility occurs at 5% or higher level.

4) Among the selected amino acids the SID of threonine decreases mostly when fiber rich components are used like wheat bran and soyhulls. The reduction in digestibility coefficient of threonine was 4.3 and 7.2%, when 100 g/kg of wheat bran and soyhulls were included, respectively.

5) The relationships between wheat bran level and SID of methionine, cystine and threonine as well as between soyhulls level of the diets and SID of lysine, methionine and cystine could be described by the linear-plateau manner. In case of including wheat bran at 30 g/kg or above level the SID of methionine and cystine, and at 68 g/kg or above level the digestibility of threonine do not decrease further. In
case of soyhulls inclusion those thresholds occur at 30 g/kg for lysine and methionine, and at 37 g/kg for cystine.

6) There is a quadratic relationship between dietary soyhulls level and SID of threonine. The quadratic equation shows that SID of threonine has a minimum value at 74 g/kg of soyhulls inclusion.

7) SID of tryptophan decreased linearly with wheat bran inclusion (P<0.05), meanwhile in case of diets included soyhulls neither linear, quadratic nor linear-plateau curve could be fitted to the plots (P>0.05).

8) In diet formulation, to maintain the digestible amino acid content of the diets, the lysine, methionine, cystine and threonine supply should be increased up to the level to compensate for the depression of SID caused by wheat bran or soyhulls inclusion, but not thereafter. According to the data in the study the lysine and methionine supplementation should not be increased above 30 g/kg soyhulls inclusion. Using wheat bran, methionine and cystine supplementation should not be increased above 30 g/kg, and threonine supplementation above 68 g/kg wheat bran inclusion.

9) The results of the study suggest that the source of NDF has to be considered in the diet formulation, since the magnitudes of the reduction in SID of amino acids are different when wheat bran and soyhulls are used.

10) Based on the research carried out it can be concluded that further studies are required to quantify the effect of different fiber sources on the standardized ileal digestibility of amino acids in pigs.
8. New scientific achievements
1) Although fiber and fat digestibility are significantly (P≤0.05) influenced by fiber x fat interaction, the digestibility of energy is principally affected by dietary fiber content. The contribution of fiber effect within the total variance of energy digestibility is 87 % when wheat bran up to 60 % and supplemental fat up to 10 % are used in diet formulation.

2) Inclusion of wheat bran and soyhulls depress the standardized ileal digestibility of amino acids with different magnitude. Whilst 2.5 % soyhulls inclusion significantly decreases the digestibility coefficients, in case of wheat bran the statistically proven reduction in digestibility occurs at 5 % or higher level.

3) The relationships between wheat bran level and SID of methionine, cystine, and threonine, as well as between soyhulls level of the diets and SID of lysine, methionine and cystine can be described by linear-plateau manner. In case of including wheat bran at 30 g/kg or above level the SID of methionine and cystine, and at 68 g/kg or above level the digestibility of threonine do not decrease further. In case of soyhulls inclusion those thresholds occur at 30 g/kg for lysine and methionine, and at 37 g/kg for cystine.
9. Summary
The use of by-products in swine nutrition will always remain important to reduce feed costs in swine production. Increasing demand for cereals in human consumption forces the animal nutritionist to use more by-product in diets of monogastric animals. The limited availability and increasing cost of energy has caused a competition between food and feed for ingredients. In the future the feed industry will have to compete not only for grain but for the by-products as well.

The process by which by-products are produced concentrates the fiber fraction. To use these ingredients the feed industry has to evaluate them precisely, considering the nutritive value of the by-products and the influence of interaction between nutrients in order to supply the nutrient requirement of livestock adequately. When having this information, the use of by-product in feed formulation will not impair the animal performance, moreover reduced feed cost can be achieved.

The behavior of different types of dietary fiber in the digestive processes depends in particular on their physico-chemical properties such as solubility and viscosity. Literary data show that soluble fiber reduces the apparent and true ileal digestibility of protein and amino acids more than insoluble fibers; however, fecal digestibility of energy is compromised by insoluble rather than soluble fiber content of the diet. It is necessary to develop regression equations that compute the reduction in ileal digestibility of nutrients as a function of different types of fiber, when fiber rich ingredients are used in diet formulation. Due to fiber-fat interactions, there may be significant differences between calculated and measured energy content of pig diets in the case of high fiber and high fat content. In order to do a precise and accurate diet formulation of a pig diet, further studies are required for the quantification of the effect of fiber-fat interaction on the digestible nutrient supply.
Therefore, the subsequent studies had the following aims:

1) to evaluate and quantify the effect of different dietary fiber and fat level on the total tract digestibility of nutrients and gross energy.

2) to determine the effect of increasing fiber content from different sources (from wheat bran and soy hulls) on the SID of selected amino acids (lysine, methionine, cystine threonine, and tryptophan).

3) to develop regression equations that can be used to predict the SID of selected amino acids applicable for different fiber sources commonly used in hog.

4) To obtain essential data for application in practical diet formulation when fiber rich components are used.

In order to achieve these aims we conducted 2 trials.

*Animals, treatments and experimental procedure of the Trial 1*

A total of 125 castrated hybrid (Large White x Landrace) growing pigs, 5 animals/treatment with no replicates with an initial body weight of 39 kg were used in the serial. The experiment was carried out with 25 dietary treatments. Pigs were fed with diets based on five different wheat bran levels (0, 150, 300, 450 and 600 g/kg) and five added fat levels (0, 25, 50, 75 and 100 g/kg) in a 5 x 5 factorial arrangement. Feed was supplied at the level of 2.6 times maintenance requirement of energy. Due to the
different energy content of the feeds, the daily feed intake was different, but
the energy intake and the ileal digestible protein, lysine, methionine, cystine
and threonine intake were the same in each treatment. A 9-day adaptation
period was followed by a 5-day collection period, during which faeces were
collected quantitatively. The animals were housed in metabolic cages during
both the adaptation and collection periods. Pigs received their diet twice
daily in mash form (1 part diet + 2 parts water) and were allowed free access
to water. Feed refusals were collected and weighed daily. Fresh faeces
production was weighed twice daily (at 8.00 a.m. and at 3.00 p.m.) and
stored below -18°C until analysis.

**Animals, treatments and experimental procedure of the Trial 2**

The trials were conducted with total of 40 castrated hybrid (Large
White x Landrace) growing pigs, 4 hybrid barrows per treatment, in 2
replicates (8 animals/treatment), with an initial mean live weight of 30 kg.
Before starting the trials the animals were fitted with PVTC-cannula. The
surgical operations were performed in accordance with van Leeuwen et al.
(1991). With this surgery operation a specially shaped T-cannula is sucked
into the cannula by vacuum generated and with the control of ileo-caecal
valve the entire digesta is voided outside. In the digestibility study 10
dietary treatments were used with increasing wheat bran (WB) or soyhulls
(SH) inclusion rates. The basal diet was a corn-soybean meal diet that was
supplemented with 0, 25, 50, 75 and 100 g/kg wheat bran (WB-0, WB-25,
WB-50, WB-75, WB-100, respectively) or soyhulls (SH-0, SH-25, SH-50,
SH-75, SH-100, respectively). The analyzed NDF content of the feeds
ranged from 135 to 167 g/kg in the wheat bran diets and from 135 to 179
g/kg in the soyhulls diets. The daily feed allowance covered 2.6 times of the
pigs’ maintenance energy requirement. The trial consisted of a 5 days
adaptation and a 3 x 12 hours collection period. The individual daily feed intake was recorded by gram precision. In the collection phases the total amount of the digesta was collected continuously (for 12 hours) according to the recommendations of Tanksley et al (1985). The volume of the collected digesta was weighed continuously and thereafter 30% of the total collected volume was freeze-dried after homogenization.

**Laboratory analysis in the trials**

The nutrient (dry matter, crude protein, crude fat, fatty acids, crude fiber, N-free extract, crude ash and NDF) and amino acid content of the feeds and the amino acid content of digesta samples were determined according to AOAC (2000). In the trial 1 the GE content of the diet and faeces was determined with adiabatic bomb calorimeter (IKA-C-4000). Prior to the experiment feed components were analyzed chemically and the formulation of experimental diets were based on laboratory results.

**Calculations and statistical analysis of trial 1**

The digestible energy content (DE) of the feeds were calculated according to Schiemann et al. (1972) as follows:

\[
DE (MJ/kg) = (24.2 \times dP) + (39.4 \times dEE) + (18.4 \times dF) + (17.0 \times dNfe)
\]

where:

- \(dP\) : digestible crude protein (g/kg)
- \(dEE\) : digestible crude fat (g/kg)
- \(dF\) : digestible crude fiber (g/kg)
- \(dNfe\) : digestible N-free extract (g/kg)

The experimental data were evaluated by means of ANOVA (SAS, 2004). For determination of the mean effect on digestibility coefficients two-ways ANOVA was used with the following general model:
\[ Y_{ijk} = \mu + A_i + B_j + (A \times B)_{ij} + e_{ijk} \]

where:

- \( Y_{ijk} \): dependent variables
- \( \mu \): overall mean
- \( A_i \): effect of fiber level, \( i=5 \) (0, 150, 300, 450, and 600 g/kg wheat bran)
- \( B_j \): effect of fat level, \( i=5 \) (0, 25, 50, 75, and 100 g/kg of added fat)
- \( (A \times B)_{ij} \): interaction of fiber and fat level
- \( e_{ijk} \): residual error

In the course of the statistical analysis, the variance analyses showed significant interaction between fiber and fat effect. Therefore, the digestibility results are presented separately and group differences at \( P \leq 0.05 \) level tested by Tukey’s test (SAS, 2004).

The contribution of the main effects (fat, fiber, fat x fiber) to the variance of the dependent variables was calculated by the VARCOMP procedure of SAS (2004). The goal of VARCOMP procedure is to estimate the contribution of each of the random effects to the variance of the dependent variable. If the replication and/or treatment x replication (T_i x R_j) interaction were not significant, the effect of replication and/or interaction was omitted from the model.

Multiple linear regressions (PROC REG) were used to predict the digestibility of nutrients and energy as a function of crude protein, crude fat, crude fiber and N-free extract (SAS, 2004).
Calculations and statistical analysis of trial 2

Standardized ileal digestibility (SID) of each amino acid was computed by the following equation, according to Jondreville et al. (1995):

\[
\text{SID} = \frac{\text{AA intake [g]} - \text{AA excretion via feces [g]} - \text{endogenous AA loss [g]}}{\text{AA intake [g]}}
\]

Where values for the endogenous AA loss was taken from a pilot study being 406, 52, 103, 592 and 204 mg/kg dry matter intake for lysine, methionine, cystine, threonine and tryptophan, respectively.

The experimental data was analyzed separately for treatments containing wheat bran and soyhulls by using a one-way ANOVA (SAS, 2004). In the case of a significant treatment effect (P<0.05) the statistical reliance of differences among treatments was verified with Tukey test (SAS, 2004). The relationship between wheat bran and soyhulls level of the diet and standardized ileal digestibility of amino acids was examined with regression analysis separately in each fiber source (SAS, 2004). The following regression analyses were carried out:

- **linear** \((Y = a_0 + a_1 X)\),
- **quadratic** \((Y = a_0 + a_1 X + a_2 X^2)\) and
- **linear-plateau with sharp transition** \((Y = a_0 - a_1^* \ln[1 + \exp(a_2 - X)];\)

where

- \(X\) is level of wheat bran or soyhulls in the diet [g/kg] and
- \(Y\) is SID of the amino acid [%].
The following conclusions can be drawn from the thesis:

1) Data from the experiment and the results of the variance component analysis show that among the factors of fiber, fat and fiber x fat interaction, the total tract digestibility of protein was mainly determined by the dietary fiber content (53 %).

2) Although fiber and fat digestibility were significantly (P≤0.05) influenced by fiber x fat interaction, the digestibility of energy was principally affected by dietary fiber content. The contribution of fiber effect within the total variance of energy digestibility was 87 % when wheat bran up to 60 % and supplemental fat up to 10 % were used in diet formulation.

3) Inclusion of wheat bran and soyhulls depress the standardized ileal digestibility of amino acids with different magnitude. Whilst 2.5 % soyhulls inclusion significantly decreases the digestibility coefficients in case of wheat bran, the statistically proven reduction in digestibility occurs at 5 % or higher level.

4) Among the selected amino acids the SID of threonine decreases mostly when fiber rich components are used like wheat bran and soyhulls. The reduction in digestibility coefficient of threonine was 4.3 % and 7.2 %, when 100 g/kg of wheat bran and soyhulls were included, respectively.

5) The relationships between wheat bran level and SID of methionine, cystine and threonine as well as between soyhulls level of the diets and SID of lysine, methionine and cystine could be described by the linear-plateau manner. In case of including wheat bran at 30 g/kg or above level the SID of methionine and cystine, and at 68 g/kg or above level the digestibility of threonine do not decrease further. In
case of soyhulls inclusion those thresholds occur at 30 g/kg for lysine and methionine, and at 37 g/kg for cystine.

6) There is a quadratic relationship between dietary soyhulls level and SID of threonine. The quadratic equation shows that SID of threonine has a minimum value at 74 g/kg of soyhulls inclusion.

7) SID of tryptophan decreased linearly with wheat bran inclusion (P<0.05), meanwhile in case of diets included soyhulls neither linear, quadratic nor linear-plateau curve could be fitted to the plots (P>0.05).

8) In diet formulation, to maintain the digestible amino acid content of the diets, the lysine, methionine, cystine and threonine supply should be increased up to the level to compensate for the depression of SID caused by wheat bran or soyhulls inclusion, but not thereafter. According to the data in the study the lysine and methionine supplementation should not be increased above 30 g/kg soyhulls inclusion. Using wheat bran, methionine and cystine supplementation should not be increased above 30 g/kg, and threonine supplementation above 68 g/kg wheat bran inclusion.

9) The results of the study suggest that the source of NDF has to be considered in the diet formulation, since the magnitudes of the reduction in SID of amino acids are different when wheat bran and soyhulls are used.

10) Based on the research carried out it can be concluded that further studies are required to quantify the effect of different fiber sources on the standardized ileal digestibility of amino acids in pigs.
New scientific achievements

1) Although fiber and fat digestibility are significantly (P≤0.05) influenced by fiber x fat interaction, the digestibility of energy is principally affected by dietary fiber content. The contribution of fiber effect within the total variance of energy digestibility is 87 % when wheat bran up to 60 % and supplemental fat up to 10 % are used in diet formulation.

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10. Összefoglalás
A takarmányozási költségek csökkentése érdekében a melléktermékek használata a sertéstakarmányozásban az elkövetkezendő időben is fontos marad. A gabonafélék humán táplálkozási célra történő növekvő felhasználása arra ösztönzi a takarmányozási szakembereket, hogy több mellékterméket használjanak a monogasztrikus állatok takarmányozásában. A korlátozottan rendelkezésre álló és egyre dráguló energiaforrások versenyhelyzetet teremtettek az élelmiszer és takarmány alapanyagokért. A jövőben a takarmányiparnak nemesak a gabonáért, de a melléktermékekért is versenyeznie kell.

A melléktermékek keletkezésük során olyan eljárások kerestekül, melyek eredményeként rosttartalmuk feldúsulhat. Annak érdekében, hogy a takarmányipar ezeket az alapanyagokat fel tudja használni, pontosan kell ismerni azok takarmányozási értékét. Ez azért rendkívül fontos, mert a komponensek táplálóértékének, valamint a táplálóanyagok közti interakcióknak az ismerete az állatok táplálóanyag igényének pontosabb kielégítését teszi lehetővé. Mindezen ismeretek birtokában lehetőség van arra, hogy a melléktermékek használatakor az állatok teljesítménye ne romoljon és a receptúrakészítés során a takarmányköltségek jelentős csökkentésével is számolni lehet.

A takarmányrost egy rendkívül heterogén anyag, az egyes rosttípusok fiziko-kémiai tulajdonságai - mint például az oldhatóság és viszkozitás - igen eltérő lehet. Irodalmi adatok azt mutatják, hogy az oldható rost, az oldhatatlanhoz képest nagyobb mértékben csökkenti az aminosavak látszólagos és a valódi ileális emészthetőségét. Ugyanakkor az energia belsából mért emészthetőségét nagyobb mértékben rontja az oldhatatlan rost, mint az oldható. A gyakorlat számára szükséges lenne olyan regressziós egyenletek kidolgozása, amellyel becsülni lehet a táplálóanyagok ileális emészthetőségének változását különböző rostforrások
esetén, amikor nagy rosttartalmú alapanyagot használunk az adag összeállításnál. Az irodalmi adatok szerint a nagy rosttartalmú takarmány zsírkiegészítésekor a zsír-rost interakció következtében szignifikáns különbség lehet a számított és a sertéstakarmányok mért energiatartalma között. A receptúrkészítés pontosságának javítása érdekében további kutatások szükségesek, melyekkel a zsír-rost interakció hatását számszerűsíteni tudjuk.

Ennek érdekében a diszsertációban ismertetésre kerülő vizsgálatok célja:
1) annak megállapítása, hogy a takarmány rost- és zsírtartalma hogyan és milyen mértékben befolyásolja a táplálóanyagok, valamint az energia bélsárból mért emészthetőségét;
2) annak meghatározása, hogy az abrákkeverék különböző rostforrása (búzakorpa és a szójahéj) miképpen befolyásolja néhány, a sertések számára fontos aminosav (lizin, metionin, treonin, triptofán) standardizált ileális emészthetőségét;
3) olyan regressziós egyenletek kidolgozása, melyek alkalmasak néhány fontosabb aminosav standardizált ileális emészthetőségének becslésére különböző sertéstakarmányozásban általánosan használt rostforrás felhasználása esetén;
4) javaslattétel nagy rosttartalmú takarmányokkal végzett vizsgálatok eredményeinek felhasználására a receptúrakészítésben.

A kitűzött cél elérése érdekében 2 kísérletet végeztünk:
**Kísérleti állatok és kezelések, állatkísérleti módszer az 1. kísérletben**

A vizsgálatokat összesen 125 hibrid (nagy fehér x lapály) ártány sertéssel végeztük, (5 állat/kezelés), melyek élősúlya a kísérlet kezdetén 39 kg volt. A kísérlet 5 x 5-ös elrendezésű volt, az állatokat a 25 kísérleti kezelés egyikébe osztottuk. Az etettek növekvő mennyiségében tartalmaztak búzakorpát (0, 150, 300, 450 és 600 g/kg) és hozzáadott zsírt (0, 25, 50, 75 and 100 g/kg). A takarmányadag a sertések életfenntartó energiaszükségletének 2,6-szeresét fedezte. Mivel a takarmányok energiatartalma különböző volt, így a napi takarmányfelvétel is különbözőtt, de a napi energiafelvétel és az ileálisan emészthető fehérje, lizin, metionin, cisztin és treonin felvétel azonos volt minden kezelésnél. A 9 napos előetetési szakaszt követően 5 napos kísérleti szakaszból a belsár teljes mennyiségét gyűjtöttük. Az állatokat anyagsere ketrecben helyeztük el mind a szoktatási, mind a gyűjtési szakaszból. A takarmányfelvételt naponta feljegyeztük, az esetleges takarmánymaradékot naponta gyűjtöttük és visszamértük. A termelődött belsarat homogenizáltuk, majd további feldolgozásig -18 °C-on tároltuk.

**Kísérleti állatok és kezelések, állatkísérleti módszer a 2. kísérletben**

Összesen 40 hibrid (nagy fehér x lapály) ártánnyal végeztük a kísérletet, ismétlésben (n=8/állat/kezelés). Az állatok induló testtömege 30 kg volt. A kísérlet megkezdése előtt az állatokat PVTC-kanüllel látottuk el. Az operáció van Leeuwen és munkatársai (1991.) módzsere szerint történt. Ezzel az operációs eljárással az ileo-cekálás billentyű által keltett vákuum miatt a speciálisan kialakított T kanülon keresztül a teljes béltartalom a külvilág felé ürül. Az emészthetőségi kísérletben 10 kezelést alakítottunk ki, a kukorica szója összetételű alaptakarmányt 0, 25, 50, 75, 100 g/kg
búzakorpával (WB-0, WB-25, WB-50, WB-75, WB-100,) vagy szójahéjjal (WB-0, SH-25, SH-50, SH-75, SH-100) egészítettük ki.

A takarmányok analizált NDF tartalma a búzakorpát tartalmazó takarmányokban 135 és 167 g/kg között, a szójahéjet tartalmazó keverékekbén 135 és 179 g/kg között változott. A takarmányadag a sertések életfenntartó energiaszükségletének 2,6-szeresét fedezte. A napi takarmányfelvételt grammyi pontossággal mértük. Az 5 napos előetetést 3 x 12 órás gyűjtési szakasz követte, melyben a béltartalom teljes mennyiségét gyűjtöttük, homogenizáltuk, majd a minta 30 %-át további feldolgozásig liofilizáltuk.

*Laboratóriumi vizsgálatok*

A takarmányok táplálóanyag tartalmát (szárazanyag, nyersfehérje, nyerszsír, zsírsav, nyersrost, nitrogénmentes kivonható anyag, nyershamu, NDF és aminosav) és a béltartalom aminosav tartalmát az AOAC (2000) ajánlása alapján mértük. Az első kísérletben a takarmány és a bélzsár bruttó energiatartalmát (GE) adiabatikus bomba kaloriméterrel (IKA-C-4000) határoztuk meg.

*Számítások és statisztikai analízis az 1. kísérletben*

A takarmányok emészthető energiatartalmát (DE) a Schiemann és mtsai. (1972) szerint számoltuk ki:

\[ DE \text{ (MJ/kg)} = (24.2 \times \text{dP}) + (39.4 \times \text{dEE}) + (18.4 \times \text{dF}) + (17.0 \times \text{dNfe}) \]

ahol:

- \(\text{dP}\) : emészthető nyersfehérje (g/kg)
- \(\text{dEE}\) : emészthető nyerszsír (g/kg)
- \(\text{dF}\) : emészthető nyersrost (g/kg)
- \(\text{dNfe}\) : emészthető nitrogénmentes kivonható anyag (g/kg)
A kísérleti adatokat variancia analízissel (SAS, 2004) elemeztük. Az emésztési együtthatók főkomponens analízise kétváltozós varancia analízissel (ANOVA) történt a következő modell alapján:

\[ Y_{ijk} = \mu + A_i + B_j + (AxB)_{ij} + e_{ijk} \]

ahol:

- \( Y_{ijk} \) : függő változó
- \( \mu \) : főátlag
- \( A_i \) : rost mennyiség hatás, \( i=5 \) (0, 150, 300, 450 and 600 g/kg búzakorpa)
- \( B_j \) : zsír mennyiség hatása, \( i=5 \) (0, 25, 50, 75 and 100 g/kg hozzáadott zsír)
- \( (A \times B)_{ij} \) : rost x zsír interakció
- \( e_{ijk} \) : maradék hiba

A függő változók főkomponens analízisét (zsír, rost, zsír x rost) variancia komponens (VARCOMP) analízissel végeztük (SAS, 2004). A VARCOMP analízis célja az volt, hogy becsülni lehessen a véletlen hatást a függő változó variációjahoz viszonyítva. A táplálóanyagok emészthetőségét többváltozós lineáris regresszió segítségével becsültük a takarmány nyersfehérje, nyerszsír, nyersrost, nitrogénmentes kivonható anyag tartalmából (SAS, 2004).

**Számítások és statisztikai analízis a 2. kísérletben**

Az aminosavak (AS) standardizált ileális emészthetőségét (SID) a következők szerint számoltuk:

\[
SID = \frac{AS \text{ felvétel [g]} - AS \text{ ürítés a bél tartalommal [g]} - \text{endogén AS ürítés [g]}}{AS \text{ felvétel [g]}}
\]
Az endogén aminosav ürítést egy előzetes kísérletben határoztuk meg. A lizin, metionin, cisztin, treonin, és triptofán esetében az endogén ürítés 406, 52, 103, 592 és 204 mg/kg szárazanyag felvételel volt.

A kísérleti adatokat egyváltozós varianciaanalízissel (SAS, 2004) értékeltek, a búzakorpa és a szójahéjat tartalmazó takarmányokat a statisztikai analízis során külön értékeltek. Az abrakkeverékek búzakorpa és szójahéj tartalma valamint az aminosavak standardizált ileális emészthetősége közti összefüggést regresszió analízissel vizsgáltuk külön rost forrásonként (SAS, 2004). A következő regresszió analíziseket végeztük el:

lineáris \( Y = a_0 + a_1 X \),

másodfokú \( Y = a_0 + a_1 X + a_2 X^2 \),

lineár-plató \( Y = a_0 - a_1 \cdot \ln[1 + \exp(a_2 - X)] \)

ahol \( X \) a búzakorpa vagy a szójahéj bekeverési aránya az adagban [g/kg]
\( Y \) az aminosavak standardizált ileális emészthetősége [%]

Az elvégzett vizsgálatokból az alábbi következtetések vonhatók le:

1) Az állatkísérletekben kapott adatok és a variancia komponens analízis eredményei azt mutatják, hogy az abrakkeverék fehérjetartalmának bélsárból mért emészthetőségét a zsír, a rost és a zsír x rost interakció tényezők közül elsősorban a takarmány nyersrost tartalma befolyásolta (53,3 %).

2) A rost és zsír bélsárból mért emészthetőségét szignifikánsan befolyásolta \( P \leq 0.05 \) a rost x zsír interakció, azonban a takarmány energiatartalmának emészthetőségére elsősorban az adag rosttartalma volt hatással. A variancia komponens analízis eredménye azt mutatta,
hogy a rost hatása az energia emészthetőségének össz-varianciájához 87 %-ban járul hozzá, amennyiben az abrakkeverék legfeljebb 60 % búzakorpát és 10 % hozzáadott zsírt tartalmaz.

3) A búzakorpa és a szójahéj takarmányba keverése eltérő mértékben csökkenti az aminosavak standardizált ileális emészthetőségét. Míg a szójahéj 2,5 %-os bekeverése már depresszív hatást fejt ki az aminosavak emészthetőségére, addig a búzakorpa esetében ez a hatás csak 5 % vagy afeletti értéknél következik be.

4) A vizsgált aminosavak közül a treonin standardizált ileális emészthetősége csökkent legnagyobb mértékben, ha olyan nagy rosttartalmú komponenseket szerepeltetünk az abrakkeverékbé, mint a búzakorpa vagy a szójahéj. 100 g/kg búzakorpa 4,3 %-kal (P≤0,05), 100 g/kg szójahéj bekeverése 7,2 %-kal (P≤0,05) csökkentette az emészthetőségi értékeket.

5) Lineár-plató összefüggés állapítható meg a búzakorpa mennyisége és metionin, cisztin, treonin valamint a szójahéj mennyisége és a lizin, metionin, cisztin standardizált ileális emészthetősége között. A metionin és cisztin esetében 30 g/kg feletti, treonin esetében 68 g/kg feletti búzakorpa bekeverése a takarmányba nem csökkenti tovább az aminosavak standardizált ileális emészthetőségét. A szójahéj bekeverésekor ezen küszöbértékek a lizin és metionin esetében 30 g/kg, a cisztin esetében 37 g/kg.

6) Az abrakkeverék szójahéjtartalma és a treonin standardizált ileális emészthetősége közötti kapcsolat másodfokú függvénnyel írható le (P≤0,05). A függvény a minimum értékét 7,4 g/kg szójahéj tartalomnál érte el.

7) Az alkalmazott kezelésekben a búzakorpának a triptofán standardizált ileális emészthetőségére gyakorolt hatása lineáris regresszióval írható le,
míg szójahéj etetésekor sem lineáris, sem másodfokú, sem pedig lineárszintű összefüggés nem volt megállapítható (P>0,05).

8) Az adag összeállításnál annak érdekében, hogy biztosítani lehessen a kívánt emészthető aminosav tartalmat, lizin, metionin, cisztin és treonin kiegészítéssel kell kompenzálni a búzakorpa és a szójahéj által okozott SID csökkenést. Adataink szerint 30 g/kg feletti szójahéj bekeverése esetén nem szükséges a lizin és metionin kiegészítés további növelése. Búzakorpa alkalmazásakor a metionin és cisztin kiegészítést 30g/kg-nál, a treonin kiegészítést 68 g/kg-nál nagyobb búzakorpa bekeverések kerülhetnek.

9) Az elvégzett vizsgálatok azt támasztják alá, hogy az NDF forrását figyelembe kell venni az adag összeállításnál, mert a búzakorpa és a szójahéj bekeverése a takarmányba különböző mértékben csökkenti az aminosavak standardizált ileális emészthetőségét.

10) Az elvégzett kutatások alapján megállapítható, hogy további vizsgálatokra van szükség, melyeknek célja annak megállapítása, hogy más rostforrások miképpen befolyásolják az aminosavak standardizált ileális emészthetőségét sertésekben.
Új tudományos eredmények:

1) A rost és zsír bélsárból mért emészthetőségét szignifikánsan befolyásolja ($P \leq 0.05$) a rost x zsír interakció, azonban a takarmány energiatartalmának emészthetőségére elsősorban az adag rosttartalma van hatással. A variancia komponens analízis eredménye azt mutatja, hogy a rost hatása az energia emészthetőségének összes-varianciájához 87 %-ban járul hozzá, amennyiben az abrakkeverék legfeljebb 60 % búzakorpát és 10 % hozzáadott zsírt tartalmaz.

2) A búzakorpa és a szójahéj takarmányba keverése eltérő mértékben csökkenti az aminosavak standardizált ileális emészthetőségét. Míg a szójahéj 2,5 %-os bekeverése már depresszív hatást fejt ki az aminosavak emészthetőségére, addig a búzakorpa esetében ez a hatás csak 5 % vagy afeletti értéknél következik be.

3) Lineár-plató összefüggés állapítható meg a búzakorpa mennyisége és metionin, cisztin, treonin valamint a szójahéj mennyisége és a lizin, metionin, cisztin standardizált ileális emészthetősége között. A metionin és cisztin esetében 30 g/kg feletti, treonin esetében 68 g/kg feletti búzakorpa bekeverése a takarmányba nem csökkenti tovább az aminosavak standardizált ileális emészthetőségét. A szójahéj bekeverésekor ezen küszöbértékek a lizin és metionin esetében 30 g/kg, a cisztin esetében 37 g/kg.
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12. References


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13. Publications from the thesis studies
Papers related to the thesis:


Oral presentations:

14. Other publications
Scientific publications


Other publications


15. Curriculum vitae
Laszlo Degen was born on 29th of September in 1958 in Tatabánya, Hungary. He graduated from the secondary education in 1977. After completing his studies in the Agricultural University, Keszthely, Faculty Mosonmagyaróvár (Hungary) he received his degree majoring in Agricultural Engineering with Animal Husbandry Specialization (MSc) in 1982. He defended his university doctor’s degree (dr. univ.) in the field of swine nutrition in the Agricultural University, Keszthely, Faculty Mosonmagyaróvár (Hungary) in 1989. In 2001 he completed the Masters of Business Administration (MBA) in Debrecen, Hungary. Having finished his business studies he has been correspondent PhD student in the University of Kaposvár, Faculty of Animal Science.

Since 1982 he worked several places and positions related to animal nutrition in research institute and feed industry. Since 1992 he has been working for Agribrands Europe Hungary, his present position is director of Technology Deployment and Quality Control.
16. Abbreviations
AA: amino acid
ADF: acid detergent fiber,
AID: apparent ileal digestibility
ANF: antinutritional factors
BW: body weight
CH: carbohydrate,
CP: crude protein,
CF: crude fiber
DCE: fecal digestibility coefficient for energy
DCP: fecal digestible crude protein,
DE: digestible energy
DEE: fecal digestible ether extract,
DM: dry matter
DMI: dry matter intake
EAAL: endogenous amino acid loss
EE: ether extract,
EHC: enzymatically hydrozed casein
ENL: endogenous nitrogen loss
FD: fecal digestibility
GIT: gastrointestinal tract
IDarg, IDhis, IDiso, IDlys, IDphe, IDtry: true ileal digestibility of Arginine, Histidine, Isoleucine, Lysine, Phenylalanine and Tryptophane, respectively,
IDF: insoluble dietary fiber,
ME: metabolisable energy,
MUFA: mono unsaturated fatty acid
MSPE: mean square prediction error
N: nitrogen
NDF: neutral detergent fiber,
NDIP: neutral detergent insoluble fiber
NE: net energy
NSP: non starch polysaccharide,
SDF: soluble dietary fiber,
SAT: saturated fatty acid
SH: soyhulls,
SI: standardized ileal
SID: standardized ileal digestibility
PUFA: poly unsaturated fatty acid
TCcp and IDcp: total tract and ileal digestibility coefficient for crude protein,
TDF: total dietary fiber,
TID: true ileal digestibility
WB: wheat bran