2 Literature Review

2.1 Qualitative Evaluation of Ileal Digesta

2.1.1 Chemical parameters

Various parameters can be employed to characterise the properties of ileal digesta and its potential changes in time, especially during weaning transition. The pH and dry matter (DM) content give information about the physical features of digesta, whereas volatile fatty acids (VFA) and lactic acid (LA) are indicative for the microbial activity of the microbial flora in the intestine. Ammonia in higher amounts is usually considered as a unfavourable metabolite, having a negative impact on the animal and the environment. The role of amines in the intestine is not yet fully elucidated. In certain amounts they are necessary for the enterocytes and their development, whereas in high amounts they are considered as detrimental.
Subsequently these parameters shall be reviewed in respect to their importance and informational value about the possible alterations in the GIT of piglets during weaning transition.

2.1.1.1 Lactic acid (LA)

Lactic acid is the main microbial metabolite present in stomach and small intestine of pigs, with lactic acid bacteria such as *Lactobacillus spp.* and *Streptococcus spp.* being the main producing microbes. These bacteria degrade the carbohydrates present in starch, non-starch polysaccharides and cell-wall components to LA. In suckling piglets lactic acid bacteria are predominant, especially those who can ferment lactose present in sow milk. After weaning the substrate for bacterial fermentation changes from mainly lactose to various carbohydrates such as fermentable carbohydrates in the starter feed. As LA is a direct metabolite detectable in the digesta, it provides an indication about possible shifts in the microbial flora and its metabolic activities. In concert with microbiological analysis one can achieve a clearer insight into the intestinal flora.
Various experiments were conducted, investigating the effect of different feed compounds and additives, especially with regard to so-called beneficial bacteria such as Lactobacilli and its metabolites. Furthermore the impact of weaning itself was a common subject in these studies. Several trials were conducted to target the critical weaning time: investigating the impact of creep-feed these authors reported a general increase in LA

postweaning, with a short-termed decline at 5 days after weaning. However, values recovered quickly and these observations coincided with a short-termed decrease in Lactobacilli (Mathew, 1994). In other studies of this group the authors reported consistently an increase in LA after weaning (Mathew, 1997; Mathew, 1998; Franklin, 2002). Results for LA varied rather between the different studies from 10 to above 200 mmol/L digesta in the terminal ileum, indicating the significance of individual factors such as age, dietary treatment and duration of the experiment.

Similar observations were also demonstrated by other groups (Canibe, 2001; Scholten, 2002) applying organic acids as feed additives in starter diets or using fermented liquid feed.

Apart from this age effect various authors demonstrated different effect of feed additives on LA production in the small intestine of weaning piglets. Blank (1998) and Canibe (2001) reported a decrease in LA concentration in the ileum in piglets receiving diets supplemented with organic acids (fumaric acid, K-diformiate). Fermented wheat in liquid feed appeared to have no or little effect on LA as demonstrated by Scholten and co-workers (2002). In contrast Højberg (2003) and colleagues reported a significant increase in LA in growing pigs fed a completely fermented liquid diet.

Williams and co-workers (2003) could demonstrate an interesting effect of fermentable carbohydrates – lactulose, wheat starch, sugar beet pulp and inulin – in weaning piglets. In the proximal and distal part of the small intestine they proofed significant higher LA concentrations for animals fed the fermentable carbohydrate diet. Furthermore, applying molecular techniques (DGGE), they showed that Lactobacilli appeared as dominant bands in the test group whereas in control animals without fermentable carbohydrates this bacterial species appeared to be much more variable. This shows nicely the value of a concerted action in applying both chemical and microbiological analysis to track changes in the microflora of pigs. Table 1 comprises recent publications dealing with LA in the small intestine of piglets.

Table 1. Lactic acid concentration in the small intestine of young pigs, differing in age and dietary treatment

Reference	Age / Live Weight	Diet	Material	Data	
Bach Knudsen 1991	40 – 50 kg	Wheat-based, differing in fibre contents	Ileal digesta	approx. 12 – 38 mmol/L	
Mathew 1994	19 – 40 d weaning: 31 d	Sow milk, corn-soy starter	Ileal digesta	prew. 96 – 118 mmol/L postw. 228 – 261 mmol/L	
Mathew 1997	20 – 41 d weaning: 21 d	Sow milk, corn-soy starter with / without galactosyl lactose	Ileal digesta	- L(+) lactate: prew. 4.85 mmol /L postw. 11.5 – 57.9 mmol/L	- D(-) lactate: prew. 3.31 mmol/L postw. 9.2 – 24.1 mmol/L
Mathew 1998	17 – 38 d weaning: 17 d	Cereal-soy starter with / without live yeast	Ileal digesta	- L(+) lactate: 11.9 mmol/L - D(-) lactate: 1.6 mmol/L	
Blank 1998	14 – 50 d weaning: 14 d	Wheat-soy meal without / with 1 %, 2 %, 3 % fumaric acid	Ileal digesta	- L(+) lactate: 30.8 mmol/100 g DM without fumaric acid 14.8 / 4.7 / 11.7 mmol/100 g DM for 1 / 2 / 3 % fumaric acid	- D(-) lactate: 15.4 mmol/ 100 g DM without fumaric acid 3.8 / 0.9 / 3.4 mmol/100 g DM for 1 / 2 / 3 % fumaric acid
Canibe 2001	35 – 57 d weaning: 28 d	Commercial starter with / without 1.8 % K-diformiate	Ileal digesta	- without K-diformiate: 24 – 44 mmol/kg OM - with K-diformiate: 13.5 mmol/kg OM	
Scholten 2002	27 – 35 d weaning: 27 d	Liquid starter with/without fermented wheat	Ileal digesta	23.7 – 29.6 mmol/L	

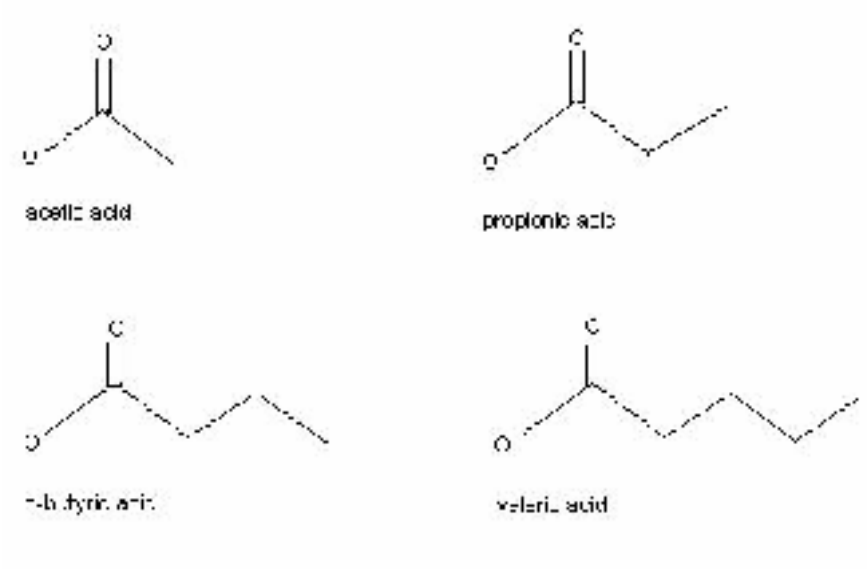
Reference	Age / Live Weight	Diet	Material	Data
Piva 2002	70 d weaning: 21 d	Cereal-soy diet without / with tributyrin or lactitol or tributyrin+lactitol	Jejunal digesta	- control: 441.9 µmol/g DM - TRB / TRB+LCT: 201.7 / 243.7 µmol/g DM - LCT: 611.2 µmol/g DM
Franklin 2002	17 – 34 d weaning: 17 vs. 24 d	Sow milk, corn-soy starter	Ileal digesta	- prew. 3.52 mmol/L L(+) lactate, 3.7 mmol/L D(-) lactate - postw. 10.4 mmol/L L(+) lactate, 6.1 mmol/L D(-) lactate
Højberg 2003	10 – 19 wk	Cereal-soy diet dry vs. fermented liquid	Ileal digesta	- dry: approx. 40 mmol/kg OM - fermented: 100 mmol/kg OM
Kamphues 2003	21 – 44 d weaning: 21 – 28 d	Pelleted commercial diet, with / without 5 % lactulose	Ileal digesta	- control: 31.9 mmol/kg OM - with lactulose: 15.5 mmol/kg OM
Williams 2003	38 d weaning: 28 d	Semi-purified control diet vs. semi-purified+fermentable carbohydrates (lactulose, inulin, wheat starch, SBP)	2nd half small intestine	- control: 3200 mg/L digesta water - fermentable carbohydrates: 4700 mg/L digesta water

2.1.1.2 Volatile Fatty Acids

Volatile fatty acids, sometimes also referred to as short chain fatty acids (SCFA), are products of microbial degradation of endogenous and exogenous material, with carbohydrates being the principal substrate. Their main representatives are acetic acid, propionic acid and butyric acid (Figure 1).

VFA exhibit various properties: they are rapidly absorbed from the gut lumen of monogastric animals, stimulate sodium and water absorption and contribute to the energy supply (Bach Knudsen, 1991), being the preferred fuel for the epithelial cells lining the GIT. The fermentation process can be observed along the entire gastrointestinal tract (GIT), varying in degree: the measurable VFA content increases from proximal to distal, reaching the highest values in the large intestine. Responsible for such a different distributional pattern are the conditions in the gut sections: the transit of digesta is generally rapid throughout the small intestine, rarely allowing accumulation at any point. Such a condition is unfavourable for a prolific microbial development and concomitant activity. Under physiological conditions the stomach and proximal half of the small intestine harbour a low number of microbes, which increases dramatically in its distal part (terminal ileum) and the large intestine (Jensen, 1998; Decuypere and van der Heyde, 1972; Canibe, 2001). Furthermore digesta is retained for a considerable time (20-38 h) in the large bowel, allowing prolific microbial activity.

Figure 1. Structure of the main representatives of volatile fatty acids



Due to these physiological conditions, research focussed mainly on VFA in the large intestine. However, as the terminal ileum harbours an already high number of resident

microbial species, one can expect a detectable amount of VFA. In the last 2 decades research displayed an increasing interest in the role of VFA in the porcine small intestine. Researchers were basically interested in the impact of age and diet, especially in young piglets. Mathew and co-workers (1994) investigated the effect of creep feed on SCFA in the ileum. Although they did not find any creep feed effect, they demonstrated a decline in SCFA shortly after weaning. Franklin (2002) examined the effect of weaning age (17d vs. 24 d) on SCFA concentration in various gut sections. Acetic acid, the main constituent of SCFA in the luminal content and taken as their representative, showed also a definite decline postweaning, regardless of weaning age. Further studies of this group comprised the investigation of the impact of different feed additives such as galactan, galactosyl lactose and live yeast culture (Mathew, 1993; Mathew, 1997; Mathew, 1998). Although in none of these studies the investigators could proof any effect of the various feed additives, they were able to demonstrate a clear time and weaning effect, respectively. Consistently they reported a distinct decline in volatile fatty acids shortly after weaning, regaining values in time but mainly not measuring up to preweaning values. This is supported by the study of Scholten and co-workers (2002), who investigated the effect of fermented wheat in liquid diets for weanling pigs. Likewise they could not demonstrate a dietary impact on VFA in the last third of the small intestine, but a decrease in VFA values postweaning and their recovery in time.

Højberg and colleagues (2003) applied an *in vitro* approach to assess the potential fermentation rate in digesta as affected by fermented liquid feed. Their investigation period comprised pigs from 10 to 17 weeks of age. While direct comparison to the previous studies is likely to be biased, they reported a general decrease in the capacity to ferment various pure substrates such as carbohydrates and carbohydrate derivates (sugar alcohols, sugar acids) in time. This was coincided with a respective decline in microbial counts of total anaerobes, lactic acid bacteria and coliforms. Therefore the authors suggested that differences in potential fermentation rate represent real differences in number or types of microorganisms and furthermore the enzymes expressed by the microbiota in time.

This *in vitro* study gives support to the previously mentioned *in vivo* results.

In contrast to this, Risley and colleagues (1992) reported an increase of VFA in time: the lowest values were demonstrated for suckling piglets and increased steadily in animals postweaning. Thus, one reason for such discrepancy might be the differential weaning age: animals in this study were weaned at 21 d of age, whereas the former studies on average weaned approximately 7 d later. However, Lactobacilli and Clostridia counts declined postweaning similar to studies reported by other authors.

Absolute values in VFA varied considerable between the different investigations, indicating the importance of standardised experimental and analytical procedures for a direct comparison. In Table 2 we give an overview of recent data on VFA in the small intestine of piglets.

Table 2. VFA in the small intestine of young pigs, differing in age and dietary treatment

Reference	Age / BW	Gut section	Experimental variables	Data	
Bolduan 1986	10 kg	Small intestine	Diets with 3 CP: - 13.2 % - 18.7 % - 24.1 %	total VFA: - 0.65 mmol/100 g OM - 1.03 mmol/100 g OM - 0.96 mmol/100 g OM	
Bach Knudsen 1991	40-50 kg	Small intestine	8 diets based on wheat-flour & wheat-fractions rich in aleurone, bran, pericarp/testa	total SCFA: 15 – 23 mmol/ L	
Risley 1992	19 – 42 d w: 21 d	Jejunum	Corn-soybean diet without additives, with 1.5 % fumaric acid, with 1.5 % citric acid	all diets: 26.0 – 43.9 Eq/dL	in time: - 6.3 mEq/dL at 19 d - 44.1 mEq/dL at 24 d - 56.6 mEq/dL at 42 d
Mathew 1993	28 – 38 d w: 28 d	Terminal ileum	Corn-soybean diet without additives, with 1 % galactan	no diet effect	in time: - 43.06 mmol/L at 28 d - 20.72 mmol/L at 30 d - 17.37 mmol/L at 38 d
Mathew 1994	19 – 40 d w: 31 d	Terminal ileum	Corn-soy diet: creep feed vs. no creep feed, identical diet as starter	no diet effect	in time (acetic acid): - 58.34 mmol/L preweaning - 25.00 mmol/L postweaning
Mathew 1997	20 – 41 d w: 21 d	Terminal ileum	Corn-soybean mash, with / without 0.5 % galactosyl lactose	no diet effect	in time (acetic acid): - 66.44 mmol/L at 20 d - 38.93 mmol/L at 30 d - 25.93 mmol/L at 41 d

Reference	Age / BW	Gut section	Experimental variables	Data	
Mathew 1998	17-38 d w: 17 d	Terminal ileum	Prestarter & starter cereal-soybean based: - pelleted - pelleted + live yeast - non-pelleted + live yeast	no diet effect	in time (acetic acid): - 38.2 mmol/L at 17 d - 22.2 mmol/L at 27 d - 30.8 mmol/L at 38 d
Canibe 2001	35 – 57 d w: 28 d	Small intestine (distal third)	Commercial starter with / without 1.8 % K-diformiate	no diet effect	acetic+propionic+butyric acid: - 4 – 8 mmol/L at 35 d - 10 – 15 mmol/L at 57 d
Scholten 2002	27 – 35 d w: 27 d	Small intestine (distal third)	Fermented vs. non-fermented wheat in liquid diet	no diet effect	in time (acetic acid): - 36.3 mmol/L at 27 d - 26.6 mmol/L at 31 d - 30.3 mmol/L at 35 d
Franklin 2002	17 – 34 d w: 17 / 24 d	Terminal ileum	Starter diet Weaning 17 d vs. 24 d	preweaning ↑VFA regardless of weaning age	In time (acetic acid): - 63.3 mmol/L at 17 d - 31.0 mmol/L at 27 d - 26.3 mmol/L at 34 d
Piva 2002	70 d w: 21 d	Jejunum	Control diet, with tributyrin, with lactitol, with tributyrin+lactitol	no diet effect total SCFA: 32.65 µmol/g DM	
Kamphues 2003	21 – 44 d w: 21 – 28 d	Small intestine (distal third)	Pelleted commercial diet, with / without 5 % lactulose	no diet effect 13.1 – 16.8 mmol/kg OM	
Højberg 2003	10 – 19 wk	Small intestine (distal third)	Fermented vs. non-fermented liquid diet	acetic+propionic+butyric acid: approx. 25 mmol/kg OM	

w: weaning time

2.1.1.3 Ammonia (NH_3)

Microbial degradation of proteins in the gastrointestinal tract produces nitrogenous metabolites that exert a rather undesirable influence on the host organism. By deamination and decarboxylation of amino acids and urea in the gut such compounds as ammonia and biogenic amines are the chief products. Both influence protein- and amino acid metabolism in the intestinal tract rather negative.

In monogastric mammals the hindgut is considered to be the main site of microbial degradation and fermentation and therefore of ammonia production. Thus research has focused mainly on this gut section in its investigation of ammonia.

But unlike to the human gut, the terminal ileum of swine shows an already dense microbial community with a considerable high metabolic activity, although not as high as in the large intestine. However, as protein degradation is considered to be nearly completed at the terminal ileum, the investigation of ammonia in the ileum seems to be of interest, especially with respect to feed protein utilisation in pigs.

Bolduan and co-workers (1986) investigated the ammonia content in the gut of piglets and sows. A striking aspect in this study is the fact that ammonia concentration increases from stomach to jejunum and again from caecum to faeces. Differences between the distal small intestine and proximal large intestine were minor: the authors reported 22.61 mmol/100 g DM in jejunal digesta and 21.16 mmol/100 g DM in caecal content of weaning piglets. Furthermore in weaning piglets they demonstrated a remarkable ability of the small intestine to deal with excess ammonia, absorbing NH_3 in large quantities via the intestinal mucosa.

Eckel and colleagues (1992) studied the effect of formic acid in varying concentrations (0.6 %, 1.2 %, 1.8 %, 2.4 %) on ammonia and biogenic amines in the intestinal tract of weaning piglets. They also demonstrated the rise of ammonia concentration along the gut from stomach to colon, but no dietary effect in the small intestine. Results were considerable lower than values by Bolduan (1986), ranging in the small intestine from 6.33 to 11.86 mmol/L digesta. They were in good agreement with data reported by Blank (1998), who also investigated the effect of organic acids on weaning piglets, although in this study fumaric acid was choosen. Similar data were published by Kamphues and colleagues (2003), studying the impact of lactulose as a feed additive in weaning and fattening pigs. Reported results were approximately 5 mmol/kg OM in the last third of the small intestine of weaners and lactulose did not exert any effect on ammonia content in these piglets.

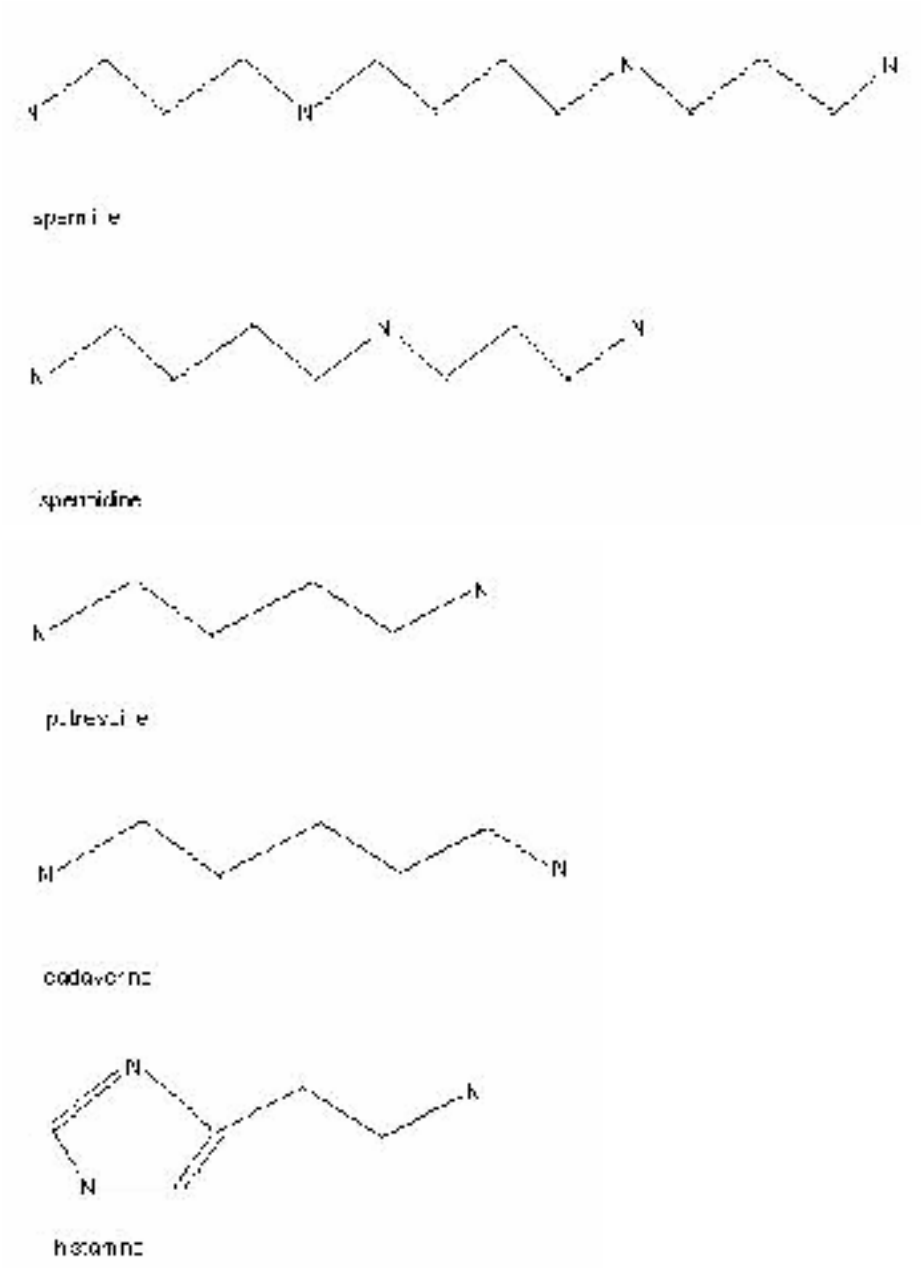
2.1.1.4 Intestinal amines

Amines are ubiquitous compounds in living organisms that participate in an array of cellular processes. They are important elements in the regulation of DNA, RNA and protein synthesis as well as cellular proliferation, differentiation and maturation (Bardocz, 1993; Blachier, 1997; Bardocz, 2001). The major amines to be found in the gastrointestinal tract of mammals are the polyamines putrescine, spermidine, spermine and the biogenic amines cadaverine and histamine (Figure 2). Putrescine is derived from ornithine under the catalysing influence of the enzyme ornithine decarboxylase (ODC) and as a precursor altered to spermidine and spermine by two synthases (spermidine and spermine synthase). Cadaverine and histamine are formed by bacterial decarboxylases from free amino acids, i.e. from lysine and histidine, respectively.

Amines detected in the gut lumen originate from different sources: dietary, endogenous and microbial amines. From the aforementioned biogenic amines only cadaverine is exclusively synthesised by prokaryotes, i.e. microbes. Putrescine, spermidine, spermine and histamine are produced by eucaryotes, i.e. enterocytes, and microbes, with the main contribution from the enterocytes. The synthesis of biogenic amines by enterocytes and bacteria seems to be dependent on the animal's age and dietary treatment.

In two elegant studies Wu and co-workers investigated the polyamine synthesis in enterocytes of suckling and weaned piglets (Wu, 2000a, b). They reported highest intracellular concentrations for spermidine, followed by putrescine and spermine. Intestinal ODC plays a key role in this synthesis. Ornithine, the immediate precursor, is negligible in sow milk and starter diets postweaning, so different amino acids have to act as metabolic sources of ornithine and therefore for polyamine synthesis as such. Wu and colleagues could identify proline in suckling and arginine in weaner piglets as the major metabolic sources. The reason for this seems to be the activity of the enzyme arginase II in the enterocytes, being very low in suckling pigs. At weaning intestinal ODC and arginase II activity increases considerably, which is most likely connected to the plasma cortisol surge during this period. Although the polyamine synthesis in the enterocytes differed, their intracellular concentration remained stable. This was partially explained by a higher uptake of dietary proline in suckling animals as sow milk is rich in proline. That underlines the crucial role of enteral provision of polyamines to maintain optimal polyamine synthesis in the intestine of young pigs.

Figure 2. Structure of amines present in the GIT of pigs



Furthermore the relationship between polyamine synthesis in the intestine and dietary and microbial impact was investigated by some authors. Wang and Higuchi (2000) investigated the impact of various diets on the mucosal polyamine production in Wistar rats. In the control animals they determined the mucosal polyamine (spermidine, spermine, acetyl spermidine) distribution along the GIT and reported highest values in duodenum, jejunum and ileum, whereas levels in the caecum and colon were low. Spermidine displayed highest intracellular levels among the polyamines in all intestinal sections. Feeding high fibre (20% beet fibre) or soy protein diets (20% soy protein isolate) resulted in a decrease of mucosal amines in the small intestine. Piva and colleagues

(2002) reported a similar decrease for histamine in jejunum and caecum of weaning piglets fed a starter (corn-soybean meal based) supplemented with 3 g/kg lactitol and 3 g/kg tributyrin. Early work of Schneider and colleagues (1989) with piglets demonstrated the dietary influence of fibre and various additives (Bisergon, 1% formic acid) on the amine concentration in the GIT. They reported a marked histamine decline in the small intestine of piglets fed diets with either 7.5 % crude fibre, 100 mg/kg Bisergon® (olanquindox) or 1% formic acid. Such dietary influence was also reported by Noack and co-workers (1998), who investigated the microbial amine synthesis in germ-free and conventional rats fed diets with different fibre sources (guar-gum, pectin). In germ-free subjects there was a basic amines level, with putrescine being the main endogenous polyamine secreted into the gut lumen and cadaverine not detectable. Alterations due to the different diets were not observed. The dietary fibres did have a marked effect on the luminal amine concentrations in conventional animals: both fibre-diets increased cadaverine concentrations dramatically and the fibre sources changed the polyamine pattern. For the guar-gum diet spermidine was predominant and for the pectin-diet cadaverine. The authors suggested that this shifts can either attributed to an alteration of bacterial metabolic activities or microbial population as such in response to the applied fibres.

Summarising these investigations give evidence for the close interrelationship between intestinal polyamine synthesis, animal age, dietary treatment and intestinal microflora. Hill and co-workers reported already 1969 about the coincidence of increasing levels of intestinal polyamines and weaning process. The above listed investigations are evidence for the recognized importance for research in this field.

2.1.1.5 D-Alanine as Bacterial Marker

Bacterial markers are a useful tool to investigate the microflora of various environments, including the gastrointestinal tract of animals. Only a part of the microbes inhabiting the GIT are cultivable, which hampers the study of this important feature in the intestine. Thus it's useful to have characteristics which are genuine to bacteria and considerable easy to analyse. In the past decades diaminopimelic acid (DAPA) was used as such a feature. This amino acid is a component of the bacterial peptidoglycan, common to gram-positive and gram-negative bacteria alike. The peptidoglycan stabilises the bacterial cell and gives it shape: its 'backbone' consists of the polysaccharides N-acetylmuramic acid and N-acetylglucosamine linked by short peptides. These peptides are generally formed by four amino acids: L-alanine, D-glutamic acid, D-alanine and diaminopimelic acid. However, in gram-positive cocci such as enterococci, L-lysine supplements diaminopimelic acid

(Schleifer & Kandler, 1972; Mendez-Alvarez, 2000). Thus, DAPA is not evenly distributed in all bacterial species, acting as a drawback in its applicability as bacterial marker.

Owing to this D-alanine was proposed as a supplemental bacterial marker, firstly applied in ruminant nutritional research (Greife, 1985; Garrett, 1987). Garrett and co-workers (1987) described the laboratory specifications for the use of D-alanine as bacterial marker extensively. They concluded that, owing to its low coefficient of variation, D-alanine is superior to most bacterial markers currently used. Schönhusen and colleagues (1995) reported similar results from their studies. They evaluated different markers such as DAPA, RNA, ^{15}N and D-alanine for their applicability in measuring microbial nitrogen passage at the duodenum of dairy cows. These authors concluded that the D-alanine method – besides the ^{15}N method - gives the most satisfactory, reproducible results for microbial protein synthesis in rumen and duodenum of dairy cows. Contradictory, Quigley and colleagues (1988) found D-alanine not a satisfactory microbial marker for calves due to a higher variation than DAPA. However, they resumed that partially this result might be attributed to the analysis of D-alanine.

In monogastric animals, especially in swine, the estimation of the microbial nitrogen fraction in the GIT is crucial in respect to the evaluation of protein metabolism. Here, like in ruminant nutrition, DAPA is commonly used as bacterial marker. Given its flaws, specifically to estimate gram-positive cocci properly, it is only logical to search for a more suitable marker. Hennig and co-workers (1999) reported the use of D-alanine as a bacterial marker in swine. D-alanine was determined in the isolated bacterial fraction of ileal digesta, given satisfactory results.

However, more research is required to evaluate and approve D-alanine as a bacterial marker in animal nutritional research.

2.1.2 Ileal Microflora

2.1.2.1 Classical Microbiological Approaches

The microbiological flora in the gastrointestinal tract of suckling and weaned piglets was investigated by numerous authors decades ago. Representative works were accomplished by Decuypere and van der Heyde (1972) and Kovacs (1972), who investigated the cultivatable luminal microflora along the intestinal sections in suckling and weaning piglets. They reported a general rise in microbial counts from proximal to distal, i.e. from stomach to colon. In the ileum the highest counts were observed for Lactobacilli (aerobic and anaerobic cultivation), followed by coliforms and Streptococci. These counts were less susceptible to changes than those in the more proximal sections like jejunum and duodenum. In fact they resembled more the microbial counts in the large intestine with the exception of Clostridia and other sporogen microorganisms, which were considerable higher in the large bowel. Furthermore those authors could demonstrate only minor numerical shifts in the ileal microbial community from pre- to postweaning period. Kovacs observed changes rather due to environmental changes (farm-reared vs. climatic chamber) than to weaning process itself. Decuypere and van der Heyde furthermore noticed a dietary influence: weaned piglets fed dry feed ad libitum displayed a similar gastrointestinal flora to suckling piglets, whereas weaners fed liquid diets restricted showed more inconsistent results and a higher susceptibility to scouring. Jensen (1998) gave an excellent review about the successive changes in the microbiota of piglets from birth to postweaning period. Lactobacilli and Streptococci were the predominant species in the small intestine during the suckling period. The author could demonstrate a short-term decrease in Lactobacilli counts and an increase in coliforms during weaning transition. About a week postweaning Lactobacilli counts recovered and coliforms declined again, displaying an inverse relationship between these two species during the first week postweaning. Additionally this author reported a considerable microbial fermentative activity in stomach and small intestine: in fact equal amounts of organic acids were produced in the three compartments stomach, small and large intestine. However, the composition of the organic acids differed, lactic acid being the major part in stomach and small intestine, whereas acetic and butyric acid contributed the main part in the large bowel. Another excellent review concerning the intestinal microbiota of piglets and influencing factors such as host characteristics, diet (composition, additives as probiotics) was given by Conway (1994).

In the subsequent years research was directed towards the investigation of various factors influencing the microbial community such as dietary treatment (physical form, additives as

antibiotics, probiotics, prebiotics, metals, organic acids etc., composition), environment (farm vs. isolator rearing), weaning (age, creep feed) and many more. One important area to examine was weaning transition and its concomitant conditions. Mathew and co-workers (1993) looked into the impact of the polysaccharide galactan on the colonisation of *E.coli* in the small intestine of weaning piglets and the possible changes in the microbial community. They could not proof any significant influence on the examined microbial population, including *E.coli*. In the subsequent years they investigated the impact of galactosyl lactose and direct-fed yeast culture on the enteric microflora in weanling pigs (Mathew, 1997; Mathew, 1998). Neither galactosyl lactose nor direct-fed yeast culture exerted any effect on the investigated microbial species Lactobacilli, Streptococci, *E.coli* and yeast. However, in the study from 1997 the author's results indicated a time-dependent response of the investigated bacterial species: during the first postweaning days *Lactobacillus spp.* decreased and *E.coli* increased numerically, but recovered quickly to preweaning values. In another experiment Mathew and colleagues (1994) analysed the impact of creep feed on the selected microflora in unweaned piglets. They did not find evidence for a significant influence of creep feed on the microbial population in the ileum. However, they did report a time-dependent impact: *Lactobacillus spp.* counts decreased and percentage of haemolytic *E.coli* (% of total *E.coli*) increased just after weaning. Such age-related observations were also made by Franklin and co-workers (2002), who investigated the impact of weaning age on the microflora (17 days vs. 24 days). Irrespective of weaning age *E.coli* population did not change and *Lactobacillus spp.* declined postweaning in both regimes. However, Lactobacilli counts in the earlier weaned group decreased to a greater extent, indicating that piglets weaned at a later stage are more adept to deal with occurring changes at weaning.

Alteration of the diet itself was investigated by Mikkelsen and Jensen (1998), who applied fermented liquid feed in weaning piglets. They could report an increase in counts of *Lactobacillus spp.* and yeast and a decline of coliforms at ileal level. A similar result was demonstrated by Scholten (2002), who fed fermented wheat to weanling pigs, although in this study there was no effect on total coliforms and *E.coli* at ileal level. Højberg and colleagues (2003) supported these findings with their study about fermented liquid feed in pigs.

Organic acids are regarded as potential supplements for in-feed antibiotics in pig nutrition. Canibe (2001a) gives an overview of the influence of organic acids on intestinal flora and subsequent impact on animal health. Studies were carried out in general in growing-finishing pigs, only to a minor proportion in weaning pigs. Risley and co-workers (1992) investigated various organic acids (1.5 % fumaric, 1.5 % citric acid) and their impact on the gut flora of weaning piglets. They could not proof any effect of the used organic acids

on the microflora, but a time-dependency of the bacteria, like in studies mentioned earlier. Contradictory Canibe (2001b) and colleagues observed a decrease of total anaerobes and lactic acid bacteria along all gut sections, but no effect on coliforms due to the inclusion of 1.8 % K-diformiate. This result indicates even an undesirable effect, as the potentially pathogenic coliforms were unaffected whereas the as beneficial regarded lactic acid bacteria were decreased. Similar findings were reported by Maribo (2000).

As pointed out previously, *Lactobacillus spp.* contributes to the main part of the microbial community in the piglet's small intestine, especially the ileum. Therefore research focussed often on this bacterial species. Naito and co-workers (1995) examined the faecal flora of piglets and their dams from birth to 6 weeks of age. They reported a change in the Lactobacilli population, with *L.acidophilus* being predominant in the first 14 days and *L.reuteri* in the successive experimental time. However, it does not reflect the ileal flora to the same extent, but rather gives an indication for possible microbial alterations. Krause and colleagues (1995) followed a different approach: they analysed the adherent *Lactobacillus spp.* population in the GIT of piglets before and after weaning. Their investigation revealed an increase in ileal total culturable counts and in *Lactobacillus spp.* postweaning compared to suckling subjects. Additionally they determined the Shannon-index of diversity for *Lactobacillus spp.* and detected the highest diversity for pigs postweaning fed solid diets (based on corn-soy-lactose) compared to piglets receiving sow milk. This indicates that the type of diet exerts a great influence on the adherent *Lactobacillus spp.* population along the gastrointestinal tract of piglets. A selection of papers dealing with the assessment of the porcine intestinal microflora by means of classical microbiological techniques is provided in Table 3.

Table 3. The culturable resident microflora in the small intestine of piglets (classical microbiology)

Reference	Technique / Material	Animal age	Variables	Observations
Decuypere & van der Heyde 1972	cultivation ileal digesta	2 – 68 d w: 1 – 16 d	- sow milk - milk replacer (liquid vs. dry, ad libitum vs. restricted)	- Lactobacilli predominant for sow milk - sow milk & ad lib-fed comparable for Lactobacilli (aerob), coliforms, enterococci
Risley 1992	cultivation jejunal digesta	19 – 42 d w: 21 d	- corn-soybean meal diets: without organic acids, with 1.5 % fumaric acid, with 1.5 % citric acid	Postweaning: ↓Lactobacilli, ↓Clostridia, ↑*E.coli*, ↑total VFA, ↑lactic acid - no effect of dietary treatments
Mathew 1993	cultivation ileal digesta	28 – 38 d w: 28 d	- corn-soybean diet with / without 1% Galactan	- ↓*E.coli* numerically for diet 1% Galactan - no difference in Lactobacilli
Mathew 1994	cultivation ileal digesta	19 – 40 d w: 31 d	- preweaning: creep feed vs. no creep feed - postweaning: corn-soy diet (18% CP, 110 mg/kg feed chlortetracycline HCl, identical with creep feed)	- preweaning: no effect of creep feed on total and haemolytic *E.coli*, Lactobacilli, VFA, pH, lactate - postweaning: ↓Lactobacilli, ↑haemolytic *E.coli* (% total *E.coli*), ↓VFA, ↑lactate for creep feed and non-creep feed animals
Krause 1995	cultivation ileal tissue	28 – 38 d w: 28 d	- sow milk (piglets remained with sow) vs. - corn-soy-lactose diet vs. - corn-soy diet	- postweaning: ↑total culturable and Lactobacilli counts in general, counts higher for dry starter diets than for sow milk - Lactobacilli diversity highest for dry starter diets
Mathew 1997	cultivation ileal digesta	20 – 41 d w: 21 d	- corn-soy-diet: with / without 0.5 % galactosyl lactose (both 19 % CP)	- postweaning: ↓Lactobacilli, short-termed ↑total *E.coli*, ↑lactate, ↓VFA - no difference between treatments
Mathew 1998	cultivation ileal digesta	17 – 38 d w: 17 d	- cereal-soy based starter with / without live yeast culture (pelleted, non-	- postweaning: ↓Lactobacilli, ↓VFA, ↑lactate, no ↑*E.coli* - no differences between diets

Reference	Technique / Material	Animal age	Variables	Observations
			pelleted)	
Jensen 1998	cultivation ileal digesta	0 – 48 d w: 28 d	- comparison of various experiments	-↓Lactobacilli, ↑coliform until 4 d postweaning - ↑Lactobacilli, ↓coliform from 7 d postweaning - adherent flora ↑effect
Mikkelsen & Jensen 1998	cultivation ileal digesta	28 – 56 d w: 28 d	- cereal-soy based liquid starter: fermented vs. non-fermented	- fermented diet: ↓coliforms, ↑yeasts, ↑lactic acid, Lactobacilli unchanged
Maribo 2000	cultivation small intestine	4 – 10 wk w: 28 d	- prestarter / starter with different percentage of organic acids (lactic, formic acid)	- lactic acid: ↑yeast and ↓coliforms - formic acid: ↓yeast - both organic acids: ↓pH in stomach and intestine, ↑organic acid production in large intestine
Canibe 2001 b	cultivation ileal digesta	35 – 57 d w: 28 d	- commercial starter with (1.8 %) / without K-diformiate	- with K-diformiate: ↓total anaerobic counts, ↓lactic acid bacteria, ↓yeasts (57d), coliforms and lactic acid unaffected
Franklin 2002	cultivation ileal digesta	17 – 34 d w: 17 d vs. 23 d	- corn-soybean meal starter - weaning 17 d vs. 24 d	- postweaning: ↓Lactobacilli, ↓acetic acid, ↑lactate irrespective of weaning age - ↑*E.coli* 3 d postweaning for 24 d weaning, no change for 17 d weaning - ↓*E.coli* from 27 d for both weaning groups
Scholten 2002	cultivation ileal digesta	27 – 35 d w: 27 d	- cereal-soy based liquid starters: with / without fermented wheat	- postweaning: ↑lactic acid bacteria, ↓total acids, ↓propionic and butyric acid - no changes for yeasts, total coliforms, *E.coli* - no differences between diets
Højberg 2003	cultivation ileal digesta	10 – 19 wk	- cereal-soy based diet: dry vs. fermented liquid feed	- fermented liquid feed: ↓coliforms, ↑lactic acid, ↓acetic, butyric and propionic acid

w: weaning time

2.1.2.2 Molecular Microbiological Approaches

In the last decade advanced molecular techniques were developed and applied in research, including the microbiological field. PCR-based techniques such as denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE) and 16S rDNA sequencing are useful tools to receive information about microbial communities and monitor possible changes. Furthermore fluorescence *in situ* hybridisation (FISH) had been used for quantification of microbial species of known sequences, providing knowledge about the actual numerical composition of the microbiota. Whereas these techniques were mostly applied in environmental research (marine vents, soil, activated sludge, wastewater etc.), in the recent past research focused on intestinal microbiota of humans and animals.

2.1.2.2.1 DGGE and Sequence Analysis

Tannock and co-workers (1990) investigated the Lactobacilli succession in the GIT of piglets by means of plasma-profiling, a DNA-based technique. They demonstrated that the Lactobacilli population in the piglet's stomach and colon were markedly different. Furthermore they found most Lactobacilli present in the sow's faeces in the piglet's GIT, indicating that maternal faeces is indeed a major source for the gut colonisation in suckling pigs. Simpson and colleagues (1999) investigated the porcine GIT by means of DGGE fingerprints in time and spatial distribution. Nursing, weaned and adult pig's faecal flora was compared and revealed marked differences in banding pattern, indicating a shift in bacterial population. Nursing piglets displayed four unique bands, whereas weaners and adults only two bands each. By comparison weaned and adult animals showed most similarities in banding pattern. Additionally the authors compared the gut sections of growing pigs. Luminal and mucosal flora (DGGE pattern) differed only marginally in the individual sections. The stomach and small intestine showed a unique PCR product undetectable in caecum and colon and vice versa PCR product singular to the large bowel could not be detected in the proximal gut sections. This results show that the intestinal bacterial flora depends on the age of the investigated subject and shows a pattern typical for the different gut sections. Another study of this group involved the administration of the *L.reuteri* strain MM53 to weaning piglets and the monitoring of successive changes in the faecal microflora by DGGE (Simpson, 2000). Their results show firstly that each animal had a highly individual and repeatable DGGE profile and secondly that the strain MM53 could be only detected during administration period and disappeared after cessation.

Studies on the large intestinal microbiota in growing pigs were conducted by Pryde and co-workers (1999), applying cloning and 16S rDNA sequencing. They reported a predominance of Lactobacilli – *L. reuteri* being the main contributor of lactic acid bacteria - and Streptococci in the identifiable sequences of luminal and mucosal bacterial isolates. A second very important finding was that a significant part of their sequences showed less than 95 % relatedness to database sequences of cultured bacteria, are therefore yet unidentifiable.

The group of Leser studied the porcine microbiota applying different fingerprint techniques, cloning and 16S rDNA sequencing (Leser, 2000; Leser, 2001). In one study they investigated the colonic flora of growing pigs in response to different diets and the experimental induction of swine dysentery with *Brachyspira hyodysenteriae*. They observed a great dietary influence on the colonic flora after a 2-week feeding, especially for diets including cooked rice, cooked rice + potato starch and cooked rice + wheat bran. For those diets they recorded the disappearance of prevotella. Furthermore they could demonstrate a destabilisation of the microbial community in the colon after experimental infection with *Brachyspira hyodysenteriae*, with the disappearance of a great proportion of bacteria and the occurrence of different bacteria undetected prior the infection. This emphasises the impact of dietary regime and infectious incidents on the intestinal microbial community in swine. In a consecutive major study Leser and co-workers (2001) investigated the GIT of pigs differing in age and dietary treatment by a cloning and sequencing approach. They could proof as well that *Lactobacillus spp.* and Streptococci seem to be the most abundant bacteria in the porcine gut. One of the most abundant Lactobacilli was *L.amylovorus*, which was here firstly isolated from the porcine gut. However, those investigators demonstrated that only 17 % of the isolates could be related to known cultured species, whereas the major contribution remains yet to be identified. Mikkelsen (2002) contributed to the molecular studies about lactic acid bacteria and investigated the genus bifidobacteria in the large intestine of suckling piglets. She reported that bifidobacteria comprise less than 1 % of the microflora in the faeces of pigs aged 1 to 4 weeks, whereas Lactobacilli (*L.reuteri*) contribute to approximately 46 %.

Konstantinov (2003) and Wei-Yun Zhu (2003) investigated the influence of fermentable carbohydrates (sugar beet pulp, oligosaccharides) on the faecal bacterial flora of weaning piglets *in vivo* and *in vitro*. Both studies showed clearly the modulation of the bacterial flora by those fermentable carbohydrates, indicating a selectively stimulation of certain strains. In the *in vitro* study inclusion of sugar beet pulp resulted in a higher similarity in the DGGE pattern between individual pigs compared to those without sugar beet pulp. The *in vivo* study clearly demonstrates a higher bacterial diversity after 2 weeks of dietary treatment, indicating a more rapid stabilisation of the microbial community after weaning.

This can be considered as beneficial, because the more stable the microbial community in such disturbing times as weaning transition the less the susceptibility for gastrointestinal disorders.

All these authors focused mainly on the microbial community in the large intestine of pigs, which does not necessarily represent the upper parts of the GIT. There are but few studies on the small intestinal flora in pigs applying molecular techniques. A recent study was conducted by Collier and colleagues (2003), investigating the response of the ileal microbiota on antimicrobial growth promoters in weaning piglets by means of DGGE and 16S rDNA sequencing. Piglets were grouped by treatment: a control without antibiotic, a group fed tylosin (40 g/ ton feed) continuously, a group with a weekly rotation of antibiotics. After 7 days no difference in band number between the groups was observed. The antibiotic treatments resulted in a suppression of the bacterial population (mainly gram-positive bacteria, e.g. Lactobacilli) compared to the control after 2 and 3 weeks of application, with the rotation system decreasing bacteria over the entire 5-weeks course. Tylosin resulted in a reestablishment of bacteria after the 3rd week, indicating a replacement of antibiotic-susceptible strains with antibiotic-resistant strains. The antibiotic treatment resulted in an increased homogeneity of the microflora, especially of the gram-positive species such as lactic acid bacteria.

The impact of certain dietary components on the resident microflora in young pigs was investigated by Konstantinov and colleagues (2004a). The authors investigated the effect of dietary fibre – supplied as fermentable carbohydrates like sugar beet pulp, wheat starch, inulin and lactulose – on the intestinal flora of weaning piglets by means of DGGE, 16S rDNA sequence analysis and FISH. They could demonstrate a significant stimulation of Lactobacilli in the ileal digesta, in particular of *L.amylovorus*, in weaned piglets fed the high fibre diet.

Table 4 gives examples of recent publications on the small intestinal microflora in piglets, investigated by means of molecular techniques.

Table 4. The resident microflora in the small intestine of piglets as revealed by molecular microbiology

References	Technique / Material	Animal age	Variables	Observations
Tannock 1990	- plasmid profling - sow faeces - piglet faeces, esophagus & stomach washings	1 – 14 d	- sow milk, different litters	- comparison sow & piglets faeces: partially Lactobacilli strains common to both, suggests sow's source for piglets microflora - *L.acidophilus* predominant in stomach days 7-14, undetected in faeces - different type strains of *L.acidophilus* - *L.fermentum* in faeces predominant at d 7-14
Simpson 1999	DGGE faeces, digesta of each gut section	18 d – 6 months w: 21 d	- sow milk - corn-soy diet	- faeces: highest similarity of banding pattern between weaners & adults, suckling pigs distinct different, ↓number of unique bands postweaning - digesta: clustering of DGGE profiles acc. to gut section
Leser 2002	16S rDNA-sequencing ileal, caecal, colonic digesta	12 – 18 wk 2, 4, 6 wk w: 28 d	- different Danish standard diets - sow milk	- most abundant phylotypes: *Eubacterium*, *Clostridium*, *Bacillus-Lactobacillus-Streptococcus* subdivision - only 17 % of detected phylotypes belonged to known bacterial species
Mikkelsen 2002	cultivation, 16S rDNA-sequencing faeces	7 – 28 d w: 28 d	- sow milk (suckling piglets)	- % of total bacterial isolates: 46 % *L.reuteri*, 4 % *L.amylovorus*, <1 % Bifidobacteria
Zhu 2003	DGGE, 16S rDNA-sequencing, faeces	41 d w: 28 d	- starter with SBP and FOS: - before vs. after *in vitro* fermentation	- after fermentation: ↑DGGE-bands (i.e. bacterial species), ↑similarity between individuals, - distinct bands in all samples: *Lachnospira pectinoschiza* (96 % similarity), *Eubacterium eligens* (92 % similarity)
Collier	DGGE, 16S rDNA-	approx. 6 – 12 wk	- corn-soybean meal diets:	- ↓total 16S rDNA bacterial population with antibiotics until 3. week

References	Technique / Material	Animal age	Variables	Observations
2003	sequencing Ileal digesta	w: unknown	antibiotic-free control, with tylosin (40 mg/kg), with weekly rotation of antimicrobials	- ↑population in tylosin group after 3 weeks, for rotation suppression remains - ↑Lactobacilli species in tylosin group vs. control and rotation
Konstantinov 2004a	DGGE, 16S rDNA-sequencing, FISH Ileal & colonic digesta	25 – 38 d w: 25 – 28 d	- starter diets: low vs. high fibre (SBP, inulin, lactulose, wheat starch)	- high fibre diet: ↑stimulation of *L.amylovorus*-like bacteria and ↑lactic acid concentration at day 10 in terminal ileum ↑diversity index in colonic digesta at day 10 - data suggest a shift in bacterial community due to dietary regime

w – weaning age

2.1.2.2.2 Fluorescence *in situ* Hybridisation (FISH)

The aforementioned molecular techniques such as DGGE are only semi-quantitative, giving information about the qualitative composition of microbial communities. However, the quantification of the identified microbial species is of great interest as well, as changes in the community might be rather numerical than qualitative.
One approach is represented by FISH. In this approach oligonucleotide probes, labelled with fluorescent dyes and aiming for typical conserved regions in different bacteria, enable the researcher to count single bacterial cells under the fluorescence microscope. An already extensive set of such oligonucleotide probes covers a wide range of different bacteria. The probes aim for different target, on genus and species level, for instance the EUB-0338 (Amann, 1990) for the domain bacteria and LAB-0722 (Sghir, 1998) for the Lactobacillus-Leuconostoc-Pediococcus cluster. By combination of different probes per sample one can obtain information about the quantity of bacterial species and their ratio to each other. In the recent years most oligonucleotide probes were applied in human nutritional research, which is reflected by the major contribution of reviews on human intestinal microbiota (Welling, 1997; Harmsen, 2002).

Summarising, molecular approaches such as DGGE, 16S rDNA sequencing and FISH are useful tools to monitor successive shifts in intestinal bacterial communities of pigs, especially in respect to the recent efforts to replace in-feed antibiotics with alternatives. Some recent reviews give an excellent overview over the state of art in molecular microbiology (Table 5).

Table 5. Reviews on recent advances in microbiological techniques and applications

Reference	Species	Techniques	Comments
O'Sullivan 2000	humans	- culture-dependent: non-selective & selective culturing - culture-independent: classical (microscopy, enzyme & metabolite analysis), molecular (16S rRNA-, ITS-, recA gene sequencing; phenotypic & genotypic fingerprinting as PFGE, DGGE; FISH)	- extensive review of classical and molecular techniques - methodology - application of techniques in investigation of intestinal microflora
Simpson 2002	swine, ruminants, salmon	DGGE	- brief overview of technique, pros & cons - swine: impact of probiotic (*L. reuteri*) on faecal flora - ruminants: ruminal flora in steers fed 2 diets (70 % corn vs. 70 % hay) - salmon: response of GIT microflora to starvation
McCartney 2002	humans	- genetic probing: FISH, dot-blot hybridisation, colony hybridisation - genetic fingerprinting: RFLP, AFLP, PFGE, ARDRA - PCR-based: RAPD, DGGE, TGGE, T-RFLP	- pro's & con's of molecular techniques for probiotic & gut flora studies - emphasis on Lactobacilli and bifidobacteria
Harmsen 2002	humans	FISH	- protocol, probes - quantification of total microflora - dynamics in human gut flora

Reference	Species	Techniques	Comments
Konstantinov 2002	humans	DGGE, TGGE, T-RFLP, DNA microarray	- DGGE: principals, analysis, interpretation, developments - specific PCR & DGGE for *Lactobacillus / Bifidobacterium* group - application for intestinal flora
Akkermans 2003	swine, humans	DGGE, 16S rDNA-sequencing	- comparison colonic & ileal flora - impact of fermentable (SBP, FOS) carbohydrates on ileal flora - comparison with human gut flora (infants, adults)
Zoetendal 2004	various (humans, swine, cattle, mice, chicken)	- 16S rDNA-sequencing - fingerprinting (DGGE, TGGE, TTGE) - quantification (RT-PCR, MPN-PCR, real time-PCR, dot blot hybridisation, FISH) - molecular approaches for metabolic activity studies of microbes	- wide array of techniques (culture-based & culture-independent) - applicability in human & animal research
Konstantinov 2004b	swine (weaning piglets)	DGGE, 16S rDNA-sequencing	- weaning-related changes in the porcine intestines - impact of fermentable carbohydrates (10 % SBP, 5 %+2.5 % SBP+FOS) on gut flora during weaning

2.2 Quantitative Evaluation of Ileal Digesta

2.2.1 Nitrogen at Ileal Level

Dietary protein is one of the key issues in commercial swine production as it provides the basis for body protein accretion in the animal. Besides protein requirement for maintenance of all bodily functions, it has a direct impact on muscle, means lean meat development. Knowledge about the fate of dietary protein provides a basis for an adequate design of diets, certainly one of the major components of commercial swine production.

From ingestion to further passage through the GIT dietary protein is subject to subsequent metabolic degradation into peptides and amino acids, which in turn are absorbed and further utilized by the animal organism. By now it is generally acknowledged that the degradation of dietary proteins is nearly fully accomplished by the end of the small intestine, i.e. the terminal ileum. Thus, in order to obtain information about protein digestion and absorption investigation has to focus on the ileal level, precisely on the terminal ileum. Nowadays researchers deal basically with three interacting concepts of protein digestibility assessments: apparent, true and real ileal digestibility of nitrogen (AID_N, TID_N, RID_N).

2.2.2 Apparent Ileal Digestibility (AID)

The simplest concept is the assessment of apparent ileal digestibility of protein. As protein is obtained by calculating nitrogen content x 6.25 and therefore nitrogen is the basic parameter to be determined for the assessment of protein digestibility, in the following we'll always refer to nitrogen digestibility – apparent ileal digestibility of nitrogen AID_N - rather than protein digestibility.

The ingested (with feed) and excreted (in faeces or digesta) amounts of nitrogen are taken into account to calculate the apparent digestibility. The basic assumption is that all nitrogen recovered in faeces or digesta originates from the feed. Secretions of other sources than dietary nitrogen (enzymes, sloughed cells and urea) along the intestinal tract during digesta passage are not included in the calculation. However, AID_N gives already a fair indication of nitrogen utilisation in the gastrointestinal tract and can be used to assess the digestibility of compound diets with various nitrogen (protein) sources.

In respect to the EU-wide ban for antimicrobial growth-promoters in weaning pigs, renewed interest has been taken in the nutritional evaluation of starter diets, varying in

their basic composition and the use of possible antimicrobial alternatives such as organic acids, fermentable carbohydrates or probiotics like lactic acid bacteria.

There are a few studies investigating the AID_N of various starters for weaning piglets. Seabra and colleagues (2001) investigated the potential use of three legume seeds – *Lupinus luteus, Vicia sativa* and *Lathyrus cicera* - as protein sources in starter diets of piglets. The legume seeds were incorporated into the diet to the expense of wheat (control diet) and ileal apparent digestibility of crude protein and amino acids determined by means of ileorectal anastomosis. The authors could demonstrate equal AID of crude protein and amino acids for the starter with *Lupinus luteus* (coefficient of AID_{CP}: 0.710) compared to the control diet (coefficient of AID_{CP}: 0.714). They stated *Lupinus luteus* a suitable supplementary protein source in diets for weaning piglets, owing to the digestibility results and the fact that the methionine+cystine and threonine pattern for this legume seed is very close to the nutritional requirements of weaning piglets. Houdijk (1999) and co-workers investigated the effect of non-digestible oligosaccharides (NDO) on digestion of weaning piglets. They reported that NDO had hardly any effect on nutrient digestion at ileal level, especially for nitrogen, compared to the highly-digestible control diet. Another study by Moeser and colleagues (2002) showed the significance of dietary fibre content on nitrogen digestion. They used dehulled, degermed corn and unaltered corn grain in diets for nursery pigs. Given, that the major part of fibre is located in the hull and germ of grain kernels, they hypothesised that removal of this fibre-rich fraction would improve nutrient digestion. Indeed, they reported a significantly enhanced AID_N for the dehulled, degermed corn diet, but no significant effect on AID of the individual amino acids. However, the authors related this to the fact that they did not correct for basal endogenous losses. Therefore they used data from literature to make the correction and calculate standardised ileal digestibility for amino acids. Results derived by this procedure showed a marked improvement in ileal digestibility of the individual amino acids, in particular for lysine, the first-limiting AA. The authors attributed their results mainly to the low fibre content of the diet containing dehulled, degermed corn. Another beneficial effect was the decreased N excretion via faeces, thus having potential to lower the negative environmental impact of commercial intensive swine production. Results to the same effect were reported by Schulze and co-workers (1994), who studied the impact of increased levels of neutral detergent fibre (NDF) on nutrient digestion in young pigs. The authors demonstrated an inverted linear relationship between elevated fibre levels and AID_N, i.e. AID_N decreasing from 88.9 % to 84.0 % with elevation of NDF levels from 0 g/kg to 180 g/kg. These findings demonstrate the importance of diet composition in weaning and growing pigs.

2.2.3 Endogenous Nitrogen and True & Real Ileal Digestibility

As mentioned earlier, the assumption that all nitrogen recovered at the terminal ileum is derived from dietary origin is not accurate. A fair amount of ileal nitrogen can be attributed to other sources than diet and was termed “endogenous nitrogen”.

Endogenous nitrogen was originally defined as the amount of nitrogen found in digesta or faeces of animals receiving nitrogen-free diets (Mitchell, 1924 cited by Souffrant, 1991). It consists of a significant amount of non-dietary nitrogen, originating from such sources like intestinal enzymes, mucus, bile and sloughed cells. In order to achieve a more precise evaluation of ileal nitrogen digestibility it is crucial to include the endogenous nitrogen losses (ENL). Based on the definition mentioned above, ENL was commonly estimated by feeding a nitrogen-free diet to animals and estimates obtained from this related to the nitrogen-containing diets applied in the respective feed evaluation. Thus, AID_N was corrected for endogenous N and true ileal digestibility of nitrogen (TID_N) calculated. However, a nitrogen-free diet represents a non-physiological condition of the animal, which might affect metabolism and therefore the reliability of the results. Furthermore, investigation of this particular subject gave evidence that endogenous N is clearly affected by the diet and its components, thus casting doubt on the nitrogen-free method (Souffrant, 1991). Efforts were made to develop more sophisticated experimental and analytical techniques to overcome this problem, thus generating techniques such as enzymatically hydrolysed protein method, homoarginine and ^{15}N-isotope method.

Darragh and Hodgkinson (2000) gave a detailed review on the topic of digestibility, endogenous N, techniques and their applicability in nutritional research. Although the paper aimed basically on human nutrition, the pig appears as the model animal, which makes their statements relevant for application in animal nutritional science as well. The authors comprised the distinctions between the digestibility concepts apparent, true and real nitrogen and amino acid digestibility (AID_N, TID_N, RID_N), based on the definitions of ENL. Whereas there’s general agreement about the term AID_N, TID_N and RID_N are often used synonymous or without a clear-cut distinction. For clarification the authors attempted to present the main differences between the two latter concepts. They distinguished between basic and specific endogenous losses, the first being diet-independent and the latter diet-dependent. Thus, the experimental method used for the estimation of endogenous N has a main impact on the resulting digestibility. Basic ENL can be estimated by either protein-free diets or EHC-method (Rutherfurd & Moughan, 1998) thus enabling the calculation of TID_N. The homoarginine method (Hagemeister & Erbersdobler, 1985) and ^{15}N-isotope technique (Souffrant, 1981) estimate the additional endogenous N – specific endogenous N - resulting from certain dietary components such protein source, fibre and anti-nutritional factors. This results in the calculation of RID_N. For the sake of

comparability between experiments it is crucial to make this distinction and thus to be able to judge possible occurring differences between results. The importance of such differentiation was demonstrated earlier by de Lange and co-workers (1990), who estimated apparent, true and real digestibility in sequential experiments, applying four different diets. One important finding was the fact that RID_N – estimated by ^{15}N-dilution technique - was always higher than TID_N (nitrogen-free method), thus indicating an underestimation of ENL by the TID_N concept. The second important result was the fact that ENL differed between the applied diets, thus highlighting the impact of dietary components on endogenous N. Chung and Baker (1992) confirmed these findings in their study, comparing nitrogen-free and nitrogen-containing diets in respect to total N excretion at ileal level. They reported a significant higher ileal N excretion at the terminal ileum for piglets fed protein-containing diets and attributed this difference to increased ENL. Apart from nitrogen dietary fibre is an important factor influencing endogenous N at ileal level. It has been demonstrated that elevated fibre levels increased endogenous N (Mariscal-Landin, 1995). Schulze and colleagues (1995) reported similar results on the inclusion of graded fibre levels. Increased fibre content enhanced the total N flow at the terminal ileum, resulting from an increase of both endogenous and exogenous N.

The problem of a dietary impact on ENL has been addressed in various studies, especially with reference to protein source, fibre source and content or antinutritional factors. Jansman and co-workers (1995), apart from investigating the mere effect of increased dietary fibre, included the effect of antinutritional factors as well. Besides confirming the fibre effect as such they could demonstrate the impact of high tannin content on endogenous N, increasing endogenous N losses additionally. Similar results were reported on the impact of lectins and phaseolin, present in white kidney beans, on endogenous N in young pigs (Schulze, 1997). A recent study carried out by Nyachoti (2000) investigated protein synthesis rates in various organs of young pigs. Pigs were fed with 2 different diets (casein-starch vs. barley), known to induce either very low or considerable high amount of ENL at the terminal ileum. Surprisingly, they did not detect changes in protein synthesis rates of the small intestine due to dietary treatment. This result indicates that at least for these specific diets, ENL can be attributed to altered endogenous N absorption rather than secretion into the ileum.

The advances in protein metabolism research – as mentioned in the above paragraphs - provided a vast body of literature on the topic of endogenous nitrogen and its influences by various factors such as age, weight and dietary regimes (Souffrant, 1991; Tamminga, 1995; Moughan, 1998; Fuller & Reeds, 1998; Mariotti, 2000; Simon, 2001; Darragh & Hodgkinson, 2000; Sève & Lahaye, 2003). These papers also provide a good source of

information about the currently applicable experimental techniques their advantages and drawbacks.

The majority of studies on ileal nitrogen digestibility (AID_N, TID_N and RID_N) and endogenous N were carried out in growing pigs. Thus, the body of information about endogenous N and TID_N / RID_N in young pigs, especially suckling and weaning piglets, is fairly limited. Another problem encountered commonly is the wide range of techniques employed in those studies, complicating further their comparability. Some studies in young pigs have already been addressed earlier in this paragraph nonetheless, table attempts to give an overview on recent digestibility studies in weaning piglets with special reference to ENL and the resulting TID_N and RID_N, respectively of various feedstuffs.

Table 6. Endogenous N and N digestibility at the terminal ileum of young pigs

Reference	Age / live weight	Technique	Variables	Data
Asche 1989	35 – 38 d	N-free diet	ad libitum vs. restricted feeding (4 x daily) of corn-soybean diet	- no effect of feeding regime on N-flow - $N_{endog.}$ 5.5 g/kg DMI
Chung & Baker 1992	10 kg BW	N-free diet	crystalline AA mix vs. casein diet	- N_{total} 2.42 g/kg DMI (AA-diet) - N_{total} 2.27 g/kg DMI (casein diet) - $N_{endog.}$ 1.56 g/kg DMI (N-free diet) - approx. 30 % of N_{total} non-proteinogen (nucleic acids, ammonia urea)
Huisman 1992	4 – 5 wk	^{15}N-leucine infusion, PVTC	2 pea varieties high tannin vs. low tannin, Phaseolus beans	- no difference between peas in $N_{endog.}$: 3.1 – 3.4 g/kg DMI - beans $N_{endog.}$: 10.7 g/kg DMI
Schulze 1995	5 – 6 wk	^{15}N-leucine infusion, PVTC	- expt.1: NDF-free vs. NDF diet (+NDF / -NDF) - expt.2: NDF-sources purified NDF (P), wheat bran (WB), sunflower hulls (SH)	expt.1: - $N_{endog.}$ 2.74 g/kg DMI (-NDF) - ↑$N_{endog.}$ 3.85 g/kg DMI (+NDF) expt.2: daily $N_{endog.}$ flow not affected by source, but ↑N_{total} for WB - ↑dietary NDF leads to ↑N_{total} due to ↑$N_{endog.}$ and ↑$N_{exog.}$
Jansman 1995	8 weeks	^{15}N-leucine infusion, PVTC	diets with highly soluble protein source: control (CT), with faba beans LT (low tannin) or faba beans HT (high tannin)	- ↑$CP_{endog.}$, ↓TID_{CP} due to faba beans - $CP_{endog.}$: 16.4 g/kg DMI (CT) 22.3 g/kg DMI (LT) 31.9 g/kg DMI (HT) - TID_{CP}: 97.2 % (CT)

Reference	Age / live weight	Technique	Variables	Data
				94.4 % (LT) 90.5 % (HT)
Schulze 1997	10 wk	^{15}N-leucine infusion, PVTC	basal diet+Phaseolus beans raw (R), germinated (G) or pancreatin-treated (P)	- ↑$CP_{endog.}$ for raw and pancreatin-treated beans - $CP_{endog.}$: 27.8 g/kg DMI (R) 13.3 g/kg DMI (G) 37.5 g/kg DMI (P)
Grala 1998	6 wk	^{15}N-leucine infusion, PVTC	- soybean / rapeseed products as sole dietary protein source - 3 soybean-products: concentrate (C), soybean meal toasted (M1), soybean meal untoasted (M2) - 3 rapeseed products: dehulled-toasted (R1), non-dehulled-toasted (R2), dehulled-untoasted (R3)	soybean products: ↑$N_{endog.}$, ↓TID_{CP} for soybean meals - $N_{endog.}$, / TID_{CP}: 2.81 g/kg DMI / 96.9 % (C) 2.53 g/kg DMI / 93.0 % (M1) 3.75 g/kg DMI / 81.1 % (M2) rapeseed products: ↑$N_{endog.}$ for R2 + R3, ↓$N_{exog.}$ for R3, ↓TID_{CP} for R1 + R2, - $N_{endog.}$, / TID_{CP}: 2.24 g/kg DMI / 85.1 % (R1) 3.03 g/kg DMI / 80.9 % (R2) 2.89 g/kg DMI / 87.8 % (R3)
Mavromichalis 2001	17 – 21 d	EHC-method, indigestible, soluble marker $YbCl_3$ / Co-EDTA, ileal T-cannula	sow milk	- $N_{endog.}$: 1201µg/g DMI (YbCL3) 1127 µg/g DMI (Co-EDTA) - TID_N: 87.8 % (average) - $TID_{AAtotal}$: 92.0 %

2.2.4 Experimental Techniques for Evaluation of Ileal Nitrogen

The most common techniques used these days for digestibility and endogenous N assessments in pigs are the ^{15}N-isotope technique (Souffrant, 1982; Simon, 1987), the EHC-method, homoarginine method (Hagemeister & Erbersdobler, 1985; Nyachoti, 1997) and nitrogen-free method. As endogenous N is commonly estimated at ileal level, these techniques have to be combined with means for digesta collection at ileal level – serial slaughter (though rarely used), cannulation of the terminal ileum (T-cannula, PVTC) or IRA.

These experimental procedures, available for the assessment of ileal nitrogen digestibility, vary in their degree of invasiveness and labour. One of the most common approaches nowadays is the cannulation of the terminal ileum in order to collect ileal digesta directly. Animals are usually confined to metabolism cages during such trials to ensure proper collection procedures. However, most cannulation techniques do not facilitate a complete quantitative collection of digesta, thus requiring the need of a marker such as chromium oxide (Cr_2O_3) as well to assess dry matter and nitrogen flow.

The only technique to date to facilitate quantitative collection of ileal digesta is the ileorectal anastomosis (IRA) as described by Hennig (1986) and Laplace (1989). It provides a powerful tool for an accurate assessment of ileal nitrogen digestibility. Briefly, animals are surgically altered by connecting the terminal ileum to the rectum, thus isolating the large intestine (Figure 3). By this means it is possible to collect ileal digesta directly and quantitatively. Different IRA techniques were developed in order to validate the least interfering and most suitable for the purpose of digestibility determination. Laplace (1994) and Redlich (1997) investigated the four main IRA forms: end-to-end and end-to side IRA with or without preservation of the ileocaecal valve. Whereas Laplace and co-workers (1994) focussed on animal growth, feed utilisation and digestibility of proteins, amino acids and carbohydrates, Redlich and colleagues (1997) investigated the impact of the morphometry of the small intestine. Both authors demonstrated that the end-to-end IRA without valve preservation represents the most suitable means in terms of maintained gut integrity and function to assess ileal digestibility. Thus, this specific IRA is nowadays applied in digestibility studies on nitrogen and amino acids.

Figure 3. Model of end-to-end ileorectal anastomosis in comparison to intact GIT