5 Discussion

5.1 Slaughter Trial

The aim of our study was the investigation and characterisation of the porcine small intestine during weaning transition and the possible influences of different starter diets. For this purpose we used German Landrace piglets of both genders before and after weaning in consecutive dietary periods: suckling period and four starters. We applied practical starter diets manufactured according to the NRC to meet the requirements of piglets.

5.1.1 Animal data

We observed the highest ADG in suckling piglets with a sharp decrease at weaning day up +2 days. Subsequent an increase in ADG was noticed, with a favour towards starter +AB until day +5. From 8 days postweaning the ADG of animals fed the home-produced diets LF and HF improved greatly and equalled or even exceeded that for +AB. The respecting daily feed intake displayed the contrary: until +5 the lowest ADFI occurred in animals fed +AB, followed by an improvement up to +15 days. The ADFI for HF equalled that of +AB at day +8 and +15. This gives an indication that at immediate weaning days the antibiotic-supplemented diet favours the zootechnical parameters ADG and ADFI, whereas the home-produced diets need an adaptation time to take effect. But already five days after weaning the parameters ameliorate for the LF and HF diet and thereafter was no difference between the antibiotic-supplemented diet and the high fibre starter. As these parameters were derived on a pen basis the conclusion regarding the dietary effects of the applied starter can give an indication only.

5.1.2 Chemical parameters

5.1.2.1 pH and DM

The pH value remained unaltered during weaning transition and was in good agreement with values demonstrated by other authors (Canibe, 2001; Mathew, 1994; Schnabel, 1985; Scholten, 2002). The DM content was significantly higher in suckling piglets and therefore decreased postweaning. Between the starter diets postweaning we did not see

any difference in DM content of ileal digesta. The postweaning values are in agreement with investigations of Bach Knudsen (1991) and Blank (1998). One explanation for the DM decrease postweaning could be the voluntary water intake. In suckling piglets the water intake is insignificant whereas with the shift to dry feed after weaning a substantial increase in water intake can be assumed. Other authors reported higher DM content in ileal digesta (Bolduan, 1986; Højberg, 2003; Kulla, 2001) of weaned piglets. The difference is likely due to the the older age and longer exposition to the dry starter feed in animals used in the studies of these authors.

5.1.2.2 Ammonia

Ammonia and amines, products of nitrogen and amino acid degradation by micro-organisms in the gut, were determined. There are only few references to be found in literature regarding these metabolites in the small intestinal digesta of weaning piglets. Eckel (1992) and Kamphues (2003) reported similar ammonia concentrations in the ileal digesta of weaning piglets as well as Blank (1998) in early-weaned piglets fed a starter diet with low buffering capacity. Eckel and co-workers (1992) studied the effect of formic acid on ammonia and amine concentration in piglets and reported values ranging between 6.33 – 11.86 mmol/L in the small intestine. Kamphues investigated the influence of lactulose on weaning and fattening pigs and demonstrated slightly lower values with approximately 5 mmol/ kg OM in the distal third of the small intestine. Values obtained by Bolduan (1986) were considerable higher, being 22.61 mmol/100 g DM (equals 22.61 mmol/kg OM) in the small intestine. Kulla (2001) and Gollnisch (1998) found lower ammonia concentrations - < 5 mmol/L ammonia - in the ileum of weaners and growing piglets. This can be attributed to the lower crude protein content in the experimental diets of these two studies, which was 18 % in contrast to 23 % CP in our own experimental diets. Furthermore Gollnisch (1998) reported a significant decrease in ammonia concentration at ileal level due to addition of avilamycin to the diet, from 3.65 ± 0.28 mmol/L to 2.74 ± 0.32 mmol/L. Although we used avilamycin at the same inclusion level in one starter diet (+AB) we could not demonstrate this effect. Ammonia concentration in pigs fed non-supplemented starters (-AB, LF, HF) was comparable to those for +AB. This suggests that in our study either avilamycin did not improve the metabolic nitrogen efficiency and therefore could not decrease ammonia or that our non-supplemented starters exert the same improvement in nitrogen efficiency. Another possibility is the impact of age and feeding duration, as animals used in Gollnisch's study were growing pigs and fed the experimental diets more than a month.

5.1.2.3 Amines

Besides ammonia or urea amines – the biogenic amines cadaverine and histamine, the polyamines putrescine, spermidine and spermine - can be classified as undesirable microbial components in the gut. The biogenic amines are end-products of bacterial proteolysis, with cadaverine derived from the amino acid lysine and histamine from histidin. Putrescine is produced by the decarboxylation of ornithine and further metabolised to spermidine and spermine. There is just little information about the content of amines in the gastrointestinal tract of food-animals, especially in swine. Schneider and co-workers (1989) investigated the amine content (histamine, putrescine, cadaverine) in the GIT of weaning and fattening pigs. Different diets were tested: high protein diet (24% CP) varying in crude fibre content (2.5, 5.0, 7.5% CF) or diet with 18% CP and different additives (1% formic acid, 100 mg/kg feed Bisergon). Values obtained for high protein diets were considerable higher than our own findings in weaning piglets. Results obtained from piglets fed the Bisergon-supplemented diet were markedly lower and comparable to our own data for histamine, putrescine and cadaverine. The amine enhancing effect of higher dietary CP could not be observed in our study as applied starters were high in CP but showed markedly lower amine content. From his study on weaning piglets Kulla (2001) reported similar data. This author determined the biogenic amines histamine and cadaverine and the polyamines putrescine, spermidine and spermine, which were in the same range as reported by Schneider (1989), i.e. higher than our amine values. Furthermore these authors noticed a high variation in amine values in groups with the same dietary treatment, a problem we encountered in our investigations as well. Due to this we could not proof any statistical difference between the starter diets used. This suggests that biogenic amines and polyamines in the gut content seem to be subjected to an individual impact of the animal rather than to a dietary impact. In his study Kulla also reported the microbial counts for *Enterococcus spp.* and *E.coli* in the caudal small intestine, which were considerable higher compared to our investigation. This suggests the possibility of stronger bacterial proteolysis and subsequently higher production of amines. Indeed we found a highly significant positive correlation between small intestinal amines and bacteria, but for the *Lactobacillus spp.* group only. Our findings suggest a strong interrelationship between specific bacterial strains and amines. From studies on food contamination it is known, that different bacterial species are able to produce amines. In food technology amines are associated with bacterial contamination and spoilage in various foods like meat and milk products. Various research groups investigated the type and amount of amines produced by bacteria like *Enterobacteriaceae*, *Lactobacillus spp.* and others. Bover-Cid and co-workers (1999, 2001) demonstrated *in vitro* that various *Lactobacillus spp.* strains produced tyramine, cadaverine, putrescine, phenylethylamine

and tryptamine, whereas *Enterobacteriaceae* produced mainly cadaverine, putrescine and to some extent histamine. Such relationship between the intestinal microflora and amine synthesis was also demonstrated in rats (Noack, 1998). This research group could proof the dependency of amine production on the existence and composition of the intestinal microbial flora. Different dietary soluble indigestible fibres (guar-gum, pectin) resulted in a changed polyamine pattern due to either an altered metabolic microbial activity or population. Porter (1970) investigated the amine production in the GI tract on weaning piglets. They reported an increase in intestinal amines immediately after weaning, with a predominance of cadaverine and putrescine. In a subsequent in-vitro study Hill (1970) could demonstrate an increase in decarboxylase and deaminative activities in the small intestinal microflora within 48 h postweaning. Those results support our results regarding the interrelationship established between polyamines and *Lactobacillus spp.*. Further studies are indeed required to elucidate the role of biogenic amines and its interrelationship with the microbial community in the gastrointestinal tract of swine.

5.1.2.4 Volatile Fatty Acids (VFA)

Total volatile fatty acids and individual SCFA (acetic, propionic, butyric, valeric, i-butyric, I-valeric acid) were measured in ileal digesta. Values were markedly lower in comparison to the large intestine, due to lower bacterial fermentation, the main factor in the production of VFA in the gut. Our results are in good agreement with data reported by Canibe et al., 2001; Bolduan et al., 1986; Kulla, 2001 and Gollnisch, 1998. Bach Knudsen (1991) investigated the microbial activity in the porcine gastrointestinal tract and demonstrated a low VFA in the ileum as well, although values were slightly higher than ours. This might be attributed to the fact that animals were older and longer exposed to solid feed. Looking at the individual SCFA, we found that mainly acetic acid contributed to the total amount of fatty acids in the ileal digesta and that contributions of propionic, butyric and valeric acid were very small. Various authors demonstrated this pattern of SCFA in the small intestine of weaning piglets (Mathew, 1994; Scholten, 2002; Franklin, 2002) as well. Furthermore Mathew (1994) and Franklin (2002) showed a decline in total VFA and acetic acid in particular after weaning compared to suckling piglets. In our study we observed this effect as well, although values did not reach statistical significance.

5.1.2.5 Lactic Acid (LA)

Lactic acid, the microbial metabolite of lactic acid bacteria, was determined to obtain a closer insight into the metabolic activity of these bacteria. Values tended to increase postweaning with a favour towards the home-produced diets, although they did not reach statistical significance. Values determined in piglets fed the home-produced diets are in accordance with results reported by Williams and co-workers (2003). This group investigated the effect of fermentable carbohydrates on small intestinal lactic acid concentrations in piglets ten days postweaning. They could demonstrate a significant increase of lactic acid in animals receiving fermentable carbohydrate-enriched diets.
The postweaning increase in lactic acid was demonstrated by various authors investigating the GIT of piglets before and after weaning (Mathew, 1994; Mathew, 1997; Franklin, 2002). Data obtained for this metabolite were in good agreement with studies investigating the gastrointestinal tract of weaning piglets (Blank, 1998; Canibe, 2001; Scholten, 2002). It seems as if this metabolite is not quite as susceptible to changes in time than others as even in older pigs (growing pigs > 30 kg BW) values are only marginally higher (Bach Knudsen, 1991; Højberg, 2003).

5.1.3 Classical Microbiology

5.1.3.1 Lactobacillus spp.

Interestingly we found that the viable *Lactobacillus spp.* counts (classical cultivation) did not alter dramatically after weaning. We noted either the same counts as preweaning or even a numerical decline after weaning. This finding is supported by the investigations of Mathew and co-workers (1994, 1997). Our *Lactobacillus spp.* counts of suckling and weaned piglets are in good agreement with these studies and with the work of Scholten (2002), and Canibe (2001) on weaned piglets. Højberg (2003) and Franklin (2002) found somewhat higher *Lactobacillus spp.* counts, which is likely due to the fact, that pigs in those studies were older or counts pooled for jejunum, ileum and caecum, respectively.
At first sight the results for *Lactobacillus spp.* counts and its metabolite lactic acid seems to be contradictory, as it was commonly accepted that those two parameters are corresponding in their performance. But already a few authors reported the *Lactobacillus spp.* decline and lactic acid increase in piglets after weaning. One possible explanation could be, that the activity of lactic acid producing bacteria itself is enhanced after weaning, rather than to attribute it to a numerical increase in bacteria. This might be due to an

altered substrate supply because of the dietary change. Another potential reason for the discrepancy in *Lactobacillus spp.* counts and lactic acid concentration is the nature of the investigated microflora. Most research – as our own investigations as well – focuses on the luminal flora and its enumeration. Savage (1977) distinguished between the luminal allochthonus (non-indigenous) and the adherent autochthonus (indigenous) flora. Actually the adherent flora might be of greater importance in the study of the nutritional impact on the GIT microbiota as it supposedly reflects changes in the microbiota indigenous to the animal. Therefore the observed lactic acid increase postweaning could be attributed to possible shifts in the adherent *Lactobacillus spp.* population rather than the luminal. This alternative view is supported by the work of Krause and co-workers (1995), who investigated the adherent *Lactobacillus spp.* population of piglets pre- and postweaning. Their results showed a distinct increase of adherent *Lactobacillus spp.* counts in the ileum in weaned piglets compared to suckling pigs. Furthermore the calculated Shannon index of diversity was higher postweaning than before weaning. This indicates that changes in the microbial flora due to dietary changes (as during weaning transition) take place in the adherent microflora rather than in the luminal flora. Further investigation is needed to elucidate the causal interrelationship between dietary supply and changes in microbial population and activity.

5.1.3.2 *Enterobacteriaceae, Enterococcus spp.* and yeast

Besides lactic acid bacteria we cultivated *Enterobacteriaceae*, *Enterococcus spp.* and yeast and counted the colony-forming units. For all cultivated bacteria, including *Lactobacillus spp.*, we observed a depression at +1 day postweaning, which recovered rapidly, usually already at +2 days. In yeasts we noted the contrary, with counts rising at +1 and dropping already at +2. These shifts were only numerically, we did not proof any statistical significance between the dietary treatments. These findings can be regarded as artefacts of the sampling as at +1 piglets had nearly no feed intake and therefore markedly less digesta in the ileum. On the other experimental days microbial counts were not statistically differential between suckling and weaning piglets and between dietary treatments. This stable state, especially of the *Enterobacteriaceae*, during weaning transition was also reflected in the piglet's health status. An intestinal overgrowth of *Enterobacteriaceae*, especially *E.coli* and *Salmonella spp.*, is one of the contributors to multi-factorial post-weaning diarrhoea, one aspect of the post-weaning growth check. During the entire experiment we did not score any incidence of post-weaning diarrhoea. Mathew and co-workers (1998) reported such a steady state of *E.coli* during weaning transition as well. Furthermore we found various studies about classically cultivated

bacteria, which support our own findings (Decuypere, 1972; Kovacs, 1972; Gedek, 1993). Only a few of these publications included yeast counts in the small intestine. Mikkelsen (1998), Canibe (2001) reported yeast counts similar to our own findings, whereas Scholten and co-workers (2002) published considerable higher values. Another interesting point was the verification of a significant negative correlation between yeast and *Lactobacillus spp.*, whereas we could not find statistical correlations between the other microbial groups. These findings suggest a close interrelationship of yeast and *Lactobacillus spp.* in the small intestine of piglets. If *Lactobacillus spp.* are present in a high amount, yeast counts are reduced. This observation is in line with the work of Maribo and co-workers (2000). They conducted experiments with piglets fed diets supplemented with lactic acid and observed a lower *Lactobacillus spp.* density and increased yeast counts along the GIT of piglets. If this interrelationship is beneficial or detrimental for the animal is not clear yet, further investigation is required.

5.1.4 Molecular Microbiology

For extended microbiological investigation we used molecular techniques such as DGGE, 16S rDNA-sequencing and FISH. Subsequently these techniques and the yielded results will be discussed.

5.1.4.1 Denaturing Gradient Gel Electrophoresis (DGGE)

DGGE profiles for the universal microflora in ileal digesta of piglets before and after weaning were quite simple with only a few dominant bands. Cluster analysis of these profiles revealed a clustering rather to age than to dietary treatment. This was supported by the assessment of the Shannon index of diversity, which showed no significant differences between the dietary treatments. Interestingly, we did observe differences between dietary treatments in the *Lactobacillus spp.*-specific DGGE profile. Already visual comparison of the profiles revealed a difference in banding pattern: there were markedly more bands in the preweaning samples than postweaning. Postweaning we noticed a decreased band number for the reference diets compared to the home-produced diets. The dendogramm showed three distinct clusters: one for the reference diets +AB, -AB, one cluster for home-produced diets LF, HF and one for suckling piglets (sow milk). Two samples for the LF and HF diets (+1 day) were not included in each of these clusters, apparently being distinctive differential from the other sample profiles. In between the cluster of reference diets the diets +AB and –AB established two sub-clusters. Not as

distinctive but visible nevertheless was the clustering in the home-produced diets. One surprising aspect of this dendogramm was the high similarity between suckling pigs and weaned piglets fed the home-produced diets. This suggests that the home-produced diets favour a *Lactobacillus spp.* population closer related to that of suckling piglets. Number of bands and Shannon index were greatest in suckling piglets. After weaning band number and diversity decreased markedly and underwent some re-adjustments until +15 days. At this time a clear distinction between the reference- and home-produced was visible, with a higher Shannon index for the home-produced diets. This supports our earlier assumption that the ileal *Lactobacillus spp.* population of animals fed LF and HF is more similar to that of suckling piglets rather than to that of pigs receiving +AB and –AB. However, to follow up these exciting results and to ensure statistical significance of it, more samples are required for *Lactobacillus spp.*-specific investigations.

In respect to the dietary impact on the Lactobacilli group, data reported by Konstantinov and colleagues (2004) are in line with our observations. The authors investigated the effect of starter diets differing in fibre contents – based on the fermentable carbohydrates lactulose, inulin, wheat starch and sugar beet pulp – on the ileal and colonic microflora in weanling pigs. By means of molecular techniques such as DGGE, sequence analysis and FISH they could demonstrate a significant stimulation of *L.amylovorus* by the high fibre diet in the luminal microflora of the ileum.

5.1.4.2 Sequence Analysis

By 16S rDNA-sequencing we were able to identify some of the dominant bands in the DGGE profiles. The phylotype OTU 171, related to the *L.amylovorus* cluster, was one of the most prevalent bacteria in ileal digesta of piglets before and after weaning as demonstrated in the DGGE profiles, universal and *Lactobacillus spp.*-specific. For the first time Leser and co-workers (2002) reported this *L.amylovorus*-like phylotype OTU 171 in the ileum and large intestine of pigs of different age and deposited in the GenBank database. Konstantinov (2003), who investigated diet-related changes in the intestinal microbiota of weaning piglets, demonstrated the high abundance of this lactobacillus, with a particular outgrowth in pigs fed diets rich in fermentable carbohydrates. Some other *Lactobacillus spp.* strains in the specific profile could be identified by comparison with *Lactobacillus spp.* clones of known sequence. Besides the *L.amylovorus*-like strain we identified *L.delbrueckii*, *L.panis*, *L.acidophilus* and *L.vaginalis* in the profile. Further identification of the remaining strains in the specific profile is still required. Another dominant band visible in the universal DGGE profile was *E.coli* (Favier, 2002), which was

only detected in samples after weaning. This particular phylotype had the highest similarity to an *E.coli* that was demonstrated in faeces of breast fed human infants.

5.1.4.3 Fluorescence *in situ* hybridisation (FISH)

By the application of the FISH technique we were able to quantify selected bacteria in the ileal digesta. General bacteria counts were obtained by use of the oligonucleotide probe EUB 0338, *Lactobacillus spp.* by the group-specific probe LAB-0722 and *E.coli* by EC 1531. At first we used two *Lactobacillus spp.* group specific probes, LAB-0158 and LAB-0722. Despite identical hybridisation procedure LAB-0158, which is generated from human origin, did not work at all in our samples, whereas the porcine-originated LAB-0722 worked well. This indicates the importance of a porcine-specific *Lactobacillus*-group probe. In our study we were able to show that the application of the *Lactobacillus* group-specific oligonucleotide probe LAB-0722 (Sghir, 1998) appears to be superior to LAB-0158 in investigating the intestinal *Lactobacillus spp.* population of weaning piglets. Using this approach we found a marked decline in general and *Lactobacillus spp.* counts postweaning, with *Lactobacillus spp.* dropping by approximately 20 % five days after weaning. This exceeded the respective results obtained by classical cultivation on MRS agar. One possible explanation is the enhanced specificity of the FISH probe compared to plate counting. By FISH one obtains a static picture of *Lactobacillus spp.*, whereas on MRS media a dynamic situation is displayed. Furthermore the selective agar plates are not as specific as FISH, so that MRS counts include not only *Lactobacillus spp.* but bifidobacteria as well, which could ameliorate the viable counts.

E.coli was below the detectable level in suckling piglets, but amounted already to 1.5×10^9 bacteria/g OM five days postweaning, regardless of the starter diet. The fact that we detected *E.coli* with DGGE and FISH only after weaning is contradictory to our results in classical cultivation techniques, where *Enterobacteriaceae* were detected already in suckling piglets. This contradiction between molecular and classical approach might be due to the fact, that the density of this bacterial group was lower than the detection limit of the applied molecular approaches ($< 10^6$ bacteria/g) before weaning, whereas with classical cultivation method colony-forming units not necessarily represent single cells but cells in a reproduction cycle (dynamic situation). Additionally this oligonucleotide probe is aiming *E.coli*, whereas on classical agar plates various bacteria belonging to the family *Enterobacteriaceae* are growing and therefore more colony-forming units can be enumerated.

5.1.5 D-alanine as Bacterial Marker

D-alanine was used as a marker for bacterial nitrogen in the digesta. D-alanine is an amino acid that only occurs in the peptidoglycan of bacterial cell walls. The peptidoglycan is a layer located between the inner and outer membrane of bacterial cells, which gives the cell shape and stability. It consists of heteropolysaccharides, connected by short peptides (L- and D-isomers of amino acids). D-amino acids, a part of these peptides, do not occur in other proteins than in bacteria. Thus it is assumed to be a suitable marker for bacterial nitrogen in the animal organism. Garret and co-workers (1987) found a low coefficient of variation and high specificity to bacteria for D-alanine in ruminants. They recommended D-alanine as superior bacterial marker in ruminants compared to many currently used markers such as diaminopimelic acid. However, so far D-alanine has hardly ever been used as bacterial marker in monogastric animals such as swine. We used the specifications of Garret (1987) and modified the protocol to suit our purpose as described in chapter 3.1.3.1. Because we aimed to determine D-alanine in digesta, without bacterial isolation, we were in need to validate the factor for this calculation. Pure bacterial cultures (*L.plantarum, Enterococcus faecium*) and bacterial isolates of ileal digesta, derived from piglets of the slaughter trial, were analysed for D-alanine content and the ratio D-alanine:N calculated. Thus we could verify that the factor required for the calculation of bacterial nitrogen was indeed alike with that used in ruminants (Schönhusen, 1995). This enabled us to estimate the bacterial nitrogen contribution in ileal digesta of piglets (unpublished data Schönhusen, Kwella). In suckling piglets the bacterial nitrogen remained quite stable, with a value of 7.82 % $\pm$ 1.74. Just after weaning the values decreased moderately in piglets fed the reference diets and markedly in animals fed home-produced diets. We noticed a steady increase in bacterial nitrogen for the reference diets until +15 days, with values exceeded preweaning data at +15d. A similar increase in time was reported by Caine and co-workers (1997), who investigated the bacterial N contribution at ileal level in newly-weaned piglets. Their results were only moderately higher than our data, despite the use of DAPA as bacterial marker. The situation for pigs fed both the home-produced diets LF and HF appeared contrary: bacterial nitrogen was always significantly lower than in suckling pigs and the aforementioned steady increase was not observed. Furthermore bacterial nitrogen was always markedly lower for LF and HF compared to both the reference diets. This shows the strong impact of weaning transition itself and a considerable dietary influence of starter diets: whereas values recovered while feeding the reference diets, this did not occur for the home-produced diets. As D-alanine is assumed to reflect the bacterial mass, our findings seem to indicate a lower bacterial mass in the ileum of pigs fed home-produced diets compared to reference diets and suckling piglets.

This relation did not correlate with the findings in microbiology, neither classical nor molecular approach. Some authors report different D-alanine results for gram-positive and gram-negative bacteria (Garrett, 1987). As the peptidoglycan in the cell wall of gram-positives amounts to 30 – 70 % whereas that of gram-negative bacteria is approximately 10 %, these authors assume that D-alanine values should be higher in gram-positives (given equal amount of gram-positive and gram-negative bacteria). Given that the small intestinal microflora of swine is dominated by gram-positive microbes one explanation for the different results could be the occurrence of more gram-positive bacteria for +AB and -AB. Microbial counts of *Lactobacillus spp.* and *Enterococcus spp.* and the genetic profile specific for *Lactobacillus spp.* show no difference between the starters, even a shift in the *Lactobacillus spp.* population in favour to the home-produced diets. However, we have no evidence about the occurrence of other gram-positive bacteria such as bifidobacteria, bacillus-Streptococci subdivision or the strict anaerobic Clostridia and eubacteria. Leser and co-workers (2002) demonstrated in their studies that the porcine gut harbours a large number of unculturable bacteria (predominantly gram-positive), which cluster phylogenetically around these species. The question of what they represent in terms of metabolic functioning is yet unanswered. Nevertheless these bacteria were possibly detected by the D-alanine method and therefore incorporated in the bacterial nitrogen results. This might have resulted in the differences between the dietary treatments. Another potential explanation is that some dietary ingredients of the home-produced interfered with the analysis, especially the legumes and wheat bran. This might lead to an underestimation of the bacterial nitrogen for these dietary treatments. Further work is required to establish the method and more reference data for swine.

5.1.6 Summary & Conclusion

Our results show that under the previously described circumstances the conditions in ileal digesta of piglets during weaning change moderately. At weaning time we could observe a general decrease in the investigated micro-organisms and their metabolites. These results are most likely connected to the low voluntary feed intake and resulting low quantity of digesta in the ileum at weaning. In general we observed changes in various parameters between suckling and weaning piglets, although these proofed scarcely statistical significant. Between the four starter diets we could see differences only in respect to bacterial nitrogen and *Lactobacillus spp.* population. However, *Lactobacillus spp.* appears to play a very important role in the ileum of piglets during weaning transition. The *L.amylovorus*-like cluster dominated the population and was consistent in its occurrence,

independent from age or dietary treatment. Apparently this group is specific for the porcine GIT as it could be only detected in content of the small and large intestine of swine (and corn silage) up to now. In a recent study Leser and co-workers (2002) reported as first of the *L.amylovorus*-like cluster in the GIT of Danish pigs differing in age and feeding regime. Furthermore we could demonstrate differential responses of the *Lactobacillus spp.* population to the dietary treatments, the home-produced diets favouring a more diverse population than the reference diets. Additionally we could detect some interesting correlations between micro-organisms and metabolites. *Lactobacillus spp.* were negative correlated with yeast and highly positive with the amines putrescine, histamine, cadaverine, spermidine and spermine. Little is known about these interrelationships in the porcine gut, especially concerning biogenic amines and their possible impact on gut physiology. Yet this is another indication for the importance of the *Lactobacillus spp.* group in the gastrointestinal tract of weaning piglets.

Another important result derived from this experiment concerned the diet effect as such. From the four starters we used in this trial we applied one diet with the in-feed antibiotic avilamycin, included as growth-promoting levels. Apart from the growth-promoting effect it is claimed to prevent gastrointestinal disorders such as diarrhoea during the immediate weaning transition. However, along the entire investigation period we could not demonstrate any beneficial antibiotic effect on the animals. None of the investigated intestinal parameters such as microbial metabolites, ileal microflora et cetera showed an alteration due to avilamycin. Furthermore we did not observe any benefits in respect to zootechnical parameters such as ADG compared to non-supplemented starters. The only impact observed occurred 15 d post-weaning, with a higher ADG in animals fed +AB compared to –AB. However, there was no obvious advantage in regard to the home-produced diets LF and HF. Therefore we conclude that the effect of avilamycin was negligible and even non-existent during the investigation period. Non-supplemented starters, especially the home-produced starters LF and HF, yielded the same effects or even better performances for parameters measured than starter +AB. One aspect to be taken into account might be the high sanitary status of the experimental facilities, which appears to play an important role at such a critical time as weaning transition. A strict hygiene regime apparently exerts a beneficial effect on animals during weaning transition, as the load of detrimental and potentially pathogenic bacteria is reduced. However, the situation in our experimental facilities does not differ a great deal from large commercial production sites nowadays, as they ensure strict sanitary sanctions in order to yield the best performance as possible. Apart from this the various stressors occurring at weaning – separation from sow and litter, environmental, social and dietary changes – and

believed to be main inducers of post-weaning growth-check, nevertheless are present and exert their impact on the piglets.

Taking this into account we expected dramatic changes in the porcine GIT. Surprisingly our investigations indicate that alterations in intestinal environment are subjected to a considerable short adaptive period. One major aspect is the apparent central role of *Lactobacillus spp.* with their numerous interrelationships in the ileal homeostatis. Further investigation is required to elucidate these aspects. Therefore we can conclude from our studies that despite the stress occurring at weaning and his quoted impact on GIT-health and performance the porcine gastrointestinal tract, i.e. at ileal level, has a great capacity to compensate and to adapt successfully to new challenges.

5.2 Balance trial pre- and postweaning

To characterise the ileum of piglets before and after weaning quantitatively two balance trials were conducted. Pre-weaning piglets were raised artificially on milk replacer, mimicking the conditions at the sow. Post-weaning animals were kept individually and fed the four starter diets applied already in the slaughter trial. Daily intake of DM, nitrogen and amino acids as well as excretion of these parameters was determined. Based on this apparent ileal digestibility of nitrogen and amino acids was calculated and by means of ^{15}N-tracer technique corrected for endogenous nitrogen. Furthermore we applied the D-alanine method to assess the contribution of microbial nitrogen.

5.2.1 Ileal flow of Dry Matter and Nitrogen

DMI was 68.4 ± 3.6 g / kg BW $^{0.75}$ in piglets in the preweaning balance trial and in weaned pigs (postweaning balance trial) 62.8 ± 5.7, 88.6 ± 7.0, 96.0 ± 6.3 and 104.8 ± 6.3 g / kg BW $^{0.75}$ for diets +AB, -AB, LF and HF, respectively. DMI in unweaned pigs was lower than in animals fed starter diets (exception +AB), with a significant lower intake compared to LF and HF. Additionally we observed a significant lower DMI in pigs receiving starter diet +AB compared to LF and HF. Mariscal-Landin and co-workers (1995) reported similar values for DMI intake, although they used growing pigs (~ 35 kg). The ileal flow of DM and nitrogen was assessed and statistical analysis revealed a significant higher DM flow in pigs fed HF. For diet LF we noticed the lowest DM flow of all starters. This can be explained by the higher ileal digestibility for this diet. Total nitrogen flow corresponded for starter LF, displaying a significantly lower value than –AB. For the high fibre diet HF total

nitrogen flow was not differential from that of diet +AB. Schulze and co-workers (1995) reported on DM and nitrogen flow at ileal level for starter diets differing in fibre content and type. They could also demonstrate a higher DM flow for diets with high fibre content.

5.2.2 Apparent Ileal Digestibility of Nitrogen and Amino Acids

Apparent ileal digestibility of nitrogen, total and individual amino acids displayed significant higher values in unweaned piglets fed milk replacer compared to weaners fed the four starter diets. Concerning the starter diets solely, we observed significant higher apparent ileal nitrogen and amino acid digestibility for diet LF. Results for AID_N are in agreement with studies from Grala (1998), Schulze (1997) and Seabra (2001). It's noteworthy that the significant higher AID_N of diet LF is similar to that obtained for starters containing soybean concentrate (Grala, 1998). This indicates that cereals and legume seeds such as sweet lupine are suitable supplements for soybean-products in starter diets. Seabra and co-workers (2001) support this with their studies on legume seeds as applicable supplements in weaning pig nutrition.

5.2.3 Endogenous Nitrogen

Endogenous nitrogen and the dietary influence on this are not taken into account by apparent ileal digestibility. However, these factors are important to assess real digestibility and with this an accurate information in respect to the feedstuff. Applying the ^{15}N tracer technique we were able to estimate endogenous nitrogen losses in pigs before and after weaning. In unweaned pigs endogenous nitrogen flow (g/d and relative to CPI or DMI) was significantly lower than in weaned pigs fed the starter diets. Comparing the starters solely revealed that endogenous nitrogen losses were highest in animals fed the high fibre diet (HF), although it did not reach statistical significance. Antinutritional factors (ANF) such as trypsin inhibitors, lectins and tannins and cell wall components (high amount of NSP) are widely made responsible for an increase in endogenous nitrogen losses and ergo decreased digestibility (Tamminga, 1995; Grala, 1998; Schulze, 1997; Jansman, 1995). ANF can be detected in both components soybean meal and legume seeds, but to a greater extent in legume seeds and NSP in legume seeds. So one might expect increased endogenous N losses and decreased digestibility, apparent and real, for the starters LF and HF. Although we observed indeed higher endogenous N contribution in those diets, the non-digested dietary N was considerable lower than in the reference diets. This indicates that despite the impact of such factors as ANF and cell wall components in legume seeds the digestion and absorption is comparable to soybean products and even

improved. Jansman (1995) investigated the effect of faba bean hulls (*Vicia faba L.*) on digestibility and endogenous protein in piglets. He reported a similar increase of endogenous N in high fibre diets. Schulze and co-workers (1995) support these findings with their studies on dietary level and fibre source in pigs. Our results for endogenous nitrogen losses in weaned piglets are largely in agreement with investigations accomplished by the authors mentioned above, Huisman (1992), Asche (1989) and for unweaned piglets by Mavromichalis (2001), although our data are slightly higher in some cases. The reason for this can be attributed first to individual differences between animals such as age, breed or genetic background and second to the different methods applied. None used the IRA technique, but merely simple T-cannulas in the terminal ileum. Furthermore the techniques for the estimation of endogenous N differed. Some studies used the method of protein-free diets or enzymatically hydrolysed proteins – Asche (1989), Mavromichalis (2001) - for the assessment of endogenous nitrogen at ileal level. Hereby the dietary impact on endogenous N losses was not taken into account – in contrast to the ^{15}N-tracer technique - and so probably underestimated. Huisman and co-workers (1992) did use the ^{15}N-technique and T-cannulas, although they infused ^{15}N-leucine intravenously in contrast to our own experiment administering labelled ^{15}N orally.

5.2.4 Real Ileal Digestibility of Nitrogen

Endogenous nitrogen estimation enabled us to calculate RID_N. RID_N was significantly higher in pigs fed milk replacer (pre-weaning condition) than in those receiving the reference starters +AB and –AB post-weaning, but there was no significant difference to home-produced LF and HF. Comparing the starter diets revealed that RID_N of the two reference diets was significantly lower than LF. Our data for weaned piglets are in good agreement with investigations by Huisman (1992), Schulze (1997). Grala and co-workers (1998) investigated the digestibility of soybean and rapeseed products. They estimated a higher real digestibility for the soybean products, especially for soybean concentrate. RID_N for soybean concentrate was similar to results obtained for our home-produced diets, whereas the lower RID_N for rapeseed products agreed with those of the reference diets. Our findings imply that from a nutritional point of view our home-produced diets, which are based on cereals and legume seeds, are equal if not superior to diets containing soybean products. Data on real digestibility in unweaned piglets are scarce. Mavromichalis (2001) determined true nitrogen digestibility (TID_N) of unweaned cannulated piglets fed sow's milk by means of enzymatically hydrolysed casein-based diet and indigestible soluble markers (co-EDTA, $YbCl_3$). They reported lower values than in our own study (92.8 % TID_N). This

can be attributed to the differences in applied methods, as the calculated TID_N rather than RID_N, thus not taking the diet-specific endogenous N losses into account.

5.2.5 Bacterial Nitrogen

Additionally we determined microbial nitrogen in the ileal content, applying the D-alanine method. Statistical comparison between pre- and postweaning period revealed significantly lower microbial nitrogen before weaning (expressed as g/100g DMI, g/100g CPI). An exception was diet LF, which did not show a significant difference to the preweaning condition. Between the starters we noticed that reference diets +AB and -AB had a higher microbial nitrogen contribution than LF and HF, with LF being significantly lower. There is but little information about bacterial nitrogen in ileal digesta in piglets around weaning. Wünsche and co-workers (1991) determined the bacterial N contribution in ileal digesta of piglets, applying different methods for digesta collection: PVTC and IRA (end-to-end or end-to-side anastomosis). They demonstrated the importance of the applied method. The end-to-end IRA is suitable for collection of ileal digesta uncontaminated with bacteria from the large intestine (reflux from caecum to ileum end-to-side IRA). The authors assessed the average bacterial nitrogen of approximately 25 % of total ileal N. They are in good agreement with studies from Schulze (1994) and Bartelt (1994). Reported data on bacterial nitrogen in ileal digesta are considerable higher than those we obtained in our studies. The main reason for this difference can be attributed to the used animals: basically these authors used growing or fattening pigs and in one case miniature pigs (Bartelt, 1994) with an average live weight well above 25 kg. It can be assumed that the bacterial N contribution in such animals is much higher than in weaning piglets with an average live weight below 10 kg. Furthermore those investigators applied DAPA (diaminopimelic acid) as bacterial marker and determined it in isolated bacterial fraction in contrast to our study, where D-alanine was determined directly in digesta. However, although applying DAPA in isolated bacterial fraction Huang (2001) reported lower values for bacterial nitrogen in ileal digesta of growing pigs, ranging between 14 and 21 % of total ileal nitrogen. Such differences between studies using the same methods indicate that there is first an age-dependent increase in bacterial mass and second a high individuality between pigs in respect to bacterial fraction in the small intestine. This assumption is supported by the investigations of Wünsche (1991).

At present the use of D-alanine as a bacterial marker is prevalent in the ruminant section, where it is regarded as superior to DAPA (Schönhusen, 1995; Garrett, 1987). There is but one short communication that refers to the use of D-alanine in pigs (Hennig, 1999). However, the authors used growing miniature and saddle back pigs with an average live

weight above 25 kg and therefore reported higher bacterial N in ileal digesta. At present literature dealing with the assessment of bacterial nitrogen contribution in the small intestine of weaning piglets is sparse. Further investigations are required in this field, especially to evaluate the use of D-alanine as a suitable bacterial marker in pigs.

5.2.6 Constitution of Total Ileal Nitrogen

As we determined both endogenous and microbial nitrogen we were able to obtain a picture of total nitrogen composition in the terminal ileum of piglets before and after weaning. Piglets fed milk replacer, starters LF and HF showed a high endogenous N fraction and only a minor part originating from dietary N sources (exogenous N). Statistical analysis showed that endogenous nitrogen (% of total N) of animals fed diet -AB was significantly lower than those fed milk replacer preweaning. Comparing starter diets solely demonstrated that endogenous N contribution in pigs receiving reference diets was lower than in those fed home-produced diets. However, differences proofed to be statistically significant for diet LF only. Bacterial nitrogen contribution was basically identical in piglets fed milk replacer and those fed the reference diets and lower for LF and HF. However, statistical analysis proofed no significant difference between animals before and after weaning, whereas comparing starters only revealed a significant lower bacterial N for home-produced diets. This implies that the relative magnitude of these two components of total nitrogen did not alter considerably during weaning transition and that endogenous N is more dependent on the applied starter diet than microbial N. The partitioning of the total ileal nitrogen reflects the digestibility of nitrogenous components in the applied dietary regimes. The occurrence of endogenous N at the terminal ileum in pigs can be attributed to various factors: pancreatic secretions (digestive enzymes, e.g. lipase as milk replacer contains a high fat level 17.7%), small intestinal secretions such as enzymes, mucins and sloughed cells (Souffrant, 1991; Tamminga 1995). These are the main contributors to endogenous N losses in swine. Looking closely at the constitution of total nitrogen at the terminal ileum provides an impression of the digestive properties of different diets. The high proportion of endogenous N in piglets fed milk replacer and home-produced diets and the considerable low amount of non-absorbed exogenous (i.e. dietary N) nitrogen emphasises their good utilisation. In contrast to this the reference diets display a slightly inverse situation, with higher exogenous and lower endogenous contributions. This indicates an impaired dietary N utilisation and a better re-absorption of endogenous N in comparison. Bacterial nitrogen contribution seems to play a minor role at such a young age. Our results demonstrated that although there were differences between starters, the overall bacterial N was very low.

5.2.7 Summary & Conclusion

In both balance trials we observed considerable differences in apparent and real ileal nitrogen digestibility, bacterial and endogenous nitrogen flow before and after weaning. In unweaned piglets we found a very high ileal nitrogen digestibility (apparent and real) of the milk replacer. Endogenous and bacterial nitrogen flow (expressed as g/100g DMI resp. CPI) was markedly lower, but as percentage of total ileal nitrogen comparable to the ileal N constitution of weaned piglets (except for –AB). Postweaning we could demonstrate striking differences between the reference and home-produced diets regarding ileal nitrogen constitution and digestibility. Both the reference diets showed a lower endogenous contribution, but higher exogenous and bacterial N contribution compared to the home-produced diets. We estimated an enhanced ileal nitrogen digestibility – apparent and real – for the home-produced diet LF, which we were not able to detect for the antibiotic supplemented diet +AB. Our investigations gave no indication of improved nitrogen digestibility, decreased ENL or bacterial N for the antibiotic-supplemented reference diet +AB. Parameters determined in course of this balance trial did not differ between the two reference diet, i.e. no beneficial effects of avilamycin-supplementation versus the non-supplemented diets (reference starter –AB and home-produced starters LF, HF) could be demonstrated.

In the contrary the best results in terms of ileal nitrogen digestibility in weaning piglets were obtained for a plain cereal-based starter without any antimicrobial and growth-promoting additives – home-produced diet LF. Yet for the cereal- and legume based starter HF, containing a high crude fibre content - AID_N and RID_N were in the same range as those for animals fed +AB with avilamycin. These results show that cereals and legume seeds are suitable components for pig starter diets and in the physical and chemical composition as used in this experiment even superior to antibiotic supplemented starters. The starter diet including in-feed antibiotic avilamycin did not show any convincing benefits compared to non-supplemented starters used in the frame of this balance experiment.

Therefore we conclude that the home-produced diet LF without in-feed antibiotic, based on cereal- and legume seeds, is an applicable starter that could be of good use in commercial swine production, particularly with regards to the EU-wide ban of antimicrobial feed additives in weaning piglets.

However, more research is required to understand underlying mechanisms of endogenous nitrogen losses in piglets pre- and postweaning and to develop possible alternatives for a gentle weaning process.