Micro-Organisms as Feed Additives – Probiotics

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Introduction

For many decades antibiotics (so called growth promoters) have been used as feed additives in various species of farm animals, to reduce the frequency of diarrhoea under certain conditions. Furthermore, in most cases performance parameters like body weight gain or feed conversion ratio improves to up to 5%. These beneficial effects of feed antibiotics are generally explained by modifications of the intestinal bacteria and their interaction with the host animal, including bacterial interactions with intestinal tissue (turnover of epithelial cells, surface coating – formation and secretion of mucins, cell invasion and resulting lesions) as well as the immune system (response of the lymphocyte population and formation and secretion of immune globulins). Thus, the intestinal microbiota is not only involved in nutrient conversion along the gastrointestinal tract, but may also affect or support animal health.

Because of the concern that the use of antibiotics as feed additive might contribute to an increase of bacterial antibiotic resistance, the European Union (EU) has decided to ban antibiotics as feed additives from 1st January 2006 onwards. Therefore, many activities were initiated to establish other substances with beneficial effects on animals via modifications of the intestinal microbiota. Among these so called “alternatives to antibiotics” are probiotics, prebiotics, organic acids and herbs, as well as essential oils.
The Use Of Microorganisms In Human And Animal Nutrition

Microbial Origin And Application

The concept of probiotics goes back to Elie Mechnikoff who proposed almost 100 years ago that bacteria in fermented milk products may be capable to control bacterial fermentation in the intestinal tract of men and thus are health promoting. Today, yoghurts containing living probiotic bacteria are available for the consumer. Bacteria with the highest potential to support human health are believed to be *Lactobacillus* and *Bifidobacterium* species.

Especially during the last decade, the concept of probiotics has been applied in animal nutrition as well. However, the use of micro-organisms in feed differs considerably from that in human food (Table 1). Out of 21 probiotic preparations actually authorised for the use as feed additives in the EU 13 are approved for the use in piglets and only some of them in feed for sows and fattening pigs. Seven of these preparations are selected strains of *Enterococcus faecium* (natural habitat: digestive tract), two contain spores of the bacterial genus *Bacillus* (natural habitat: soil), two are strains of the *Saccharomyces cerevisiae* yeast (natural habitat: fruits) and only one product contains *Lactobacillus farcininis* and *Pediococcus acidilactici*, which habitat is the digestive tract and milk products, respectively. The recommended concentration for most probiotics is approximately $10^9$ colony forming units per kg of feed.

Table 1. Probiotics in human and animal nutrition

<table>
<thead>
<tr>
<th></th>
<th>Human nutrition</th>
<th>Animal nutrition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goal</td>
<td>Long term effects</td>
<td>Quick response</td>
</tr>
<tr>
<td>Effectiveness</td>
<td>Difficult to assess</td>
<td>Easy to assess</td>
</tr>
<tr>
<td>Characteristics of intake</td>
<td>In combination with a small portion of food</td>
<td>As additive in mixed feed</td>
</tr>
<tr>
<td>Frequency of intake</td>
<td>Once per day or more?</td>
<td>10 to 20 times per day</td>
</tr>
<tr>
<td>Microorganisms (most frequent)</td>
<td><em>Lactobacillus spp.</em></td>
<td><em>Enterococcus faecium</em></td>
</tr>
<tr>
<td></td>
<td><em>Bifidobacterium spp.</em></td>
<td><em>Bacillus spp.</em></td>
</tr>
<tr>
<td></td>
<td><em>Enterococcus spp.</em></td>
<td><em>Saccharomyces cerevisiae</em></td>
</tr>
<tr>
<td>Natural habitat</td>
<td>Digestive tract, milk products</td>
<td>Digestive tract, soil, fruits</td>
</tr>
</tbody>
</table>
This has some implications for the comparison of human and animal probiotics:

- Micro-organisms used as feed additives are of different microbial origin.
- The majority of authorised probiotics are approved for the use in piglets feed.
- Most probiotics for piglets are selected strains of *Enterococcus faecium*.

**Required Properties Of Micro-Organisms For In Feed Application**

Most commonly, probiotics are defined as viable micro-organisms which, after sufficient oral intake, lead to beneficial effects for the host by modifying the intestinal microbiota. Thus, the probiotic strain must reach the intestine in a viable form and in sufficient numbers. This requires the survival of the probiotic during feed processing, including pelleting by heat in many applications, its stability in feed storage over weeks and finally its safe passage through the adverse low pH conditions in the stomach. The reason for the minor role of lactobacilli and bifidobacteria as feed additives is their poor stability during feed processing and storage. The by far most stable probiotic strains are *Bacillus* spores, because their spores are heat resistant and stay viable during long-term storage. For instance, the recovery of *Bacillus cereus toyoi* after pelleting at 87°C was 95 % and after 8 weeks in feed storage was 92 %. Vegetative, dehydrated cells like *E. faecium* are more sensitive to heat treatment (Figure 1) and inactivation during storage for 8 weeks is approximately 50 %.

**Figure 1. Recovery of viable counts of an *Enterococcus faecium* probiotic after pelleting at various temperatures** (Temperature measured in the conditioner)
By means of confection (absorption into globuli, coating) vegetative cells can be stabilized to some extent. Once the percentage loss of viability is known it can be compensated for by overdosing the initial concentrations. It is relatively easy to assess the above mentioned stability parameters. It is much more difficult to identify viable non-sporing probiotic bacteria in the intestinal tract. Especially intestinal bacteria as Enterococci or lactobacilli cannot be distinguished on the strain level with routine microbiological methods. However, with some probiotic strains this can be achieved by specific RNA or DNA probes, as will be demonstrated later.

- **Effectiveness and Mode of Action of Probiotics**

**Effectiveness**

Since probiotics are discussed as alternatives to growth promoters one should suppose they will promote animal performance. As summarized from several published experiments (Figure 2) this seems to be true as a trend for the majority of published experiments, however, the improvements were only significant in some feeding trials. This points towards a high variation in the response of the individual animals to this type of feed additive. It should be kept in mind that probiotics do not act like essential nutrients. There is no dose response, but rather a threshold level. Probiotics act mainly via modifications of intestinal bacterial populations and their effectiveness depends on the microbial status of a flock of animals and of the individual animal. Therefore, high variabilities of the effects are understandable.

Diarrhoea is the main problem for piglets during the first weeks after weaning and consequently reduction of the incidence of diarrhoea by probiotics has been studied most frequently. Published data on this aspect indicate that in approximately 80% of the experiments a significant incidence of diarrhoea was observed in piglets receiving probiotics. This effect was independent of the type of micro-organism (*B. cereus*, *E. faecium*, *P. acidilactici*). This agrees well with our experience at our experimental station, where in 3 separate studies with piglets (two with *E. faecium* NCIMB 10415, one with *B. cereus* toyoi) a significantly reduced incidence of diarrhoea was recorded, but effects on growth performance were not significant. The described situation above would at least imply less required veterinary interventions, which may be cost-saving for the producer and therefore a further aspect of efficacy.
Figure 2. Relative effects (% of control animals) of probiotics on weight gain and feed conversion in piglets (evaluations of 22 publications)

Mode of Action of Probiotics

Having in mind the use of micro-organisms of different origin with similar beneficial effects, it seems logical that their modes of action will probably be based on more than one principle. However, the modification of the microbial population seems to be the prime mode of action. The interaction between the probiotic strain and the intestinal microbiota may be based on aggregation with pathogenic bacteria, competitive adhesion to epithelial receptors, production of specific substances (organic acids, bacteriocins, dipicolinic acid), or competition for nutrients. As probably secondary effects, (primary effects of some probiotics seem also to exist) modifications of the structure and function of the intestinal epithelium as well as of the immune response were described. Although, in both fields, human and animal nutrition, great efforts have been made to study the mode of action of probiotics, our knowledge based on hard experimental data is still rather limited. The situation with regard to human nutrition was recently characterized in Nature (Abbott, 2004) as follows “…even when probiotics seem to work…..we know too little about the normal gut ecosystem to understand why”.

* significant
Within an interdisciplinary research group, including animal nutrition, microbiology, transport physiology, morphometry/histology and immunology (Project sponsored by the German Research Foundation) we have tried to study the effects of one probiotic strain (E. faecium NCIMB 10415) on various parameters. In this project the probiotic was fed to the sows during gestation and lactation and to piglets during the suckling period with creep feed and after weaning at day 28 for 6 weeks. Control animals received the same unsupplemented diets.

The results (effects of the probiotic treatment) of this integrative study can be summarised as follows:

- Performance of sows and piglets was not influenced significantly.
- Incidence of diarrhoea was reduced significantly after weaning.
- Precaecal digestibility of amino acids was significantly increased.
- No structural modifications of the epithelial tissues were observed by means of morphometry.
- Transepithelial movement of glucose in the small intestine was stimulated.
- The probiotic strain was found in the intestinal tract of piglets before offering creep feed, i.e. transfer from sow to piglet occurred.
- Bacterial communities of sows and piglets are modified and less diverse compared to controls.
- Decline of frequency of β-haemolytic and O 141 serovars of E. coli but not of total coliform bacteria in colon content of piglets.
- Significantly reduced level of cytotoxic T-cells (CD8+) in the jejunal epithelium of piglets.

**Conclusions**

From these results it may be concluded that the studied strain modifies the microbiota of sows and piglets with beneficial effects for the host animal, which were in the present experiment not reflected in the animals performance. Secondary or direct functions of epithelial tissues and immunological parameters were modified by the probiotic. Therefore, probiotics must be considered as an potential element in a feeding and management system of pig production without the use of antibiotic feed additives.

Whether or not these finding on the mode of action of E. faecium NCIMB 10415 can be applied to other micro-organisms has to be proved in further studies.
Reference