

Pre - and Probiotics: Potentials for Equine Practice

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Prebiotics and probiotics have been the subject of an increasing interest in humans as well as in animals, due to their potential positive impact on health and wellbeing. In horse feeding, numerous pre- and probiotics are available, and many are marketed for prevention or treatment of intestinal microflora disruption. However, investigations on their effects have been and are currently conducted for some of these nutritional products. There is a lack of proper research study data for the majority of them. There is no doubt that the present European regulation supports more information to be available about the use of these products.

After a presentation of the definition and the European regulation of prebiotics and probiotics, this paper will review the effects of these nutritional products in horses and discuss their potential interests for equine practice.

Definition and EU regulation

Prebiotic compounds are *selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host wellbeing and health* (Gibson, Probert et al. 2004). This updated definition extends the effect of prebiotics to the whole digestive tract while it was firstly localised *in the colon* exclusively (Gibson and Roberfroid 1995).

Inulin-type fructans ($\beta(2-1)$ fructans) such as inulin and oligofructose also named fructo-oligosaccharide (FOS), lactulose and transgalacto-oligosaccharides (TOS) are the three oligosaccharides with demonstrated prebiotic properties (Gibson, Probert et al. 2004). Manno-oligosaccharides (MOS), generally derived from outer cell wall of *Saccharomyces cerevisiae*, are other prebiotic sugars.

Prebiotic compounds were classified by the Commission in July 2005 as specific feed materials and subjected to the regulation of the Council Directive 82/471/EEC of 30 June 1982 (OJEC L213 of 21.07.1982). They require the following characteristics:

- ❖ No danger to human and animal health.
- ❖ No claim possible on efficacy. A feed material may indicate certain characteristics or properties that should be formulated carefully on the basis of objective and quantifiable parameters. The information must not contribute to mislead the user for example by attributing properties that fall within the scope of actions related to medicinal products.
- ❖ All products are generic.

Probiotic: the term means "for life". It has evolved through the years depending on different investigators and has been critically discussed. I will suggest the following definition, published by an Expert Consultation at a meeting convened by the FAO/WHO in October, 2001: "probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host". They are also known as digestive bioregulators or direct-fed microbials.

Today a large range of defined strains of probiotics belong to the group of lactic acid bacteria (*Bifidobacterium*, *Lactobacillus*, *Streptococcus*, *Enterococcus* and *Lactococcus* species), *Bacillus* sp., fungi (*Aspergillus* sp.) and yeasts (*Saccharomyces* sp., *Kluyveromyces* sp.).

Probiotics are under the control of Regulation (EC) 1831/2003 of 22 September 2003 on additives for use in animal nutrition (OJEC L268 of 18.10.2003). Probiotics are classified as Zootechnical Additives which is one of the five categories and functional groups¹ established by Regulation (EC) 1831/2003, stated as Digestibility enhancers, Gut flora stabilizers, Substances which favourably affect the environment and Others.

Only probiotics that have been through an authorisation procedure complying with the Regulation (EC) 1831/2003 may be placed on the market. Authorisations are granted for specific animal species, specific conditions of use and for ten years periods.

A probiotic shall not:

- ❖ have an adverse effect on animal health, human health or the environment,
- ❖ be presented in a manner which may mislead the user,
- ❖ harm the consumer by impairing the distinctive features of animal products or mislead the consumer with regard to the distinctive features of animal products.

A probiotic shall prove that it can:

- ❖ Favourably affect the performance of animals in good health, particularly by affecting the gastro-intestinal flora or digestibility of feedstuffs.

In equine practice, make sure the nutritional products you recommend meet the obligations drawn by the regulation. The authorisation of use is given on the strain and the animal species or type basis.

Zootechnical effects

The interaction of **prebiotics** with the intestinal microflora was proven to result in better zootechnical performance of pigs, broilers and laying hens (Flickinger, Van Loo et al. 2003; Yusrizal and Chen 2003; Chen and Chen 2005). In horses, to date, no data have reported the impact of prebiotics on growth, lactation or other technical parameters.

Most studies on probiotics were conducted with *Saccharomyces cerevisiae* (SC).

Zootechnical effects such as the improvement of milk quality in mares or of growth in foals, have been demonstrated (Glade 1991; Glade 1991). This is mainly explained by the fact that SC increases the digestive utilisation of the dietary energy. The impact of SC on digestibility has been reported in about fifteen articles (Glade and Biesik 1986; Glade and Sist 1988; Hall, Jackson et al. 1990; Pagan 1990; Glade 1991; Glade 1991; Kim, Kim et al. 1991; Glade 1992; Hausenblasz, Szuco et al. 1993; Moore and Newman 1994; Hill and Gutsell 1998; Medina 2003). Data vary according to the strain of *Saccharomyces cerevisiae*, the quantity supplemented to the horse and the composition of the ration. SC strain CBS 493.94 showed benefits for fibre digestibility of cellulose and hemicelluloses. The positive effect of SC supplementation on total cell-wall fibre (NDF) digestibility increases when the proportion of concentrate decreases in the ration. An increase in the digestibility of phosphorus indicated also a positive effect of SC on cecal fermentation (Pagan 1990).

In equine practice, *Saccharomyces cerevisiae* can be supplemented for gestating or lactating mares and for foals or growing horses in order to increase fibre digestibility.

¹ The four other categories are 1) Technological Additives, 2) Sensory Additives, 3) Nutritional Additives and 4) Coccidiostats and histomonostats

Clinical effects

In a population of 126 horses supplemented with oligofructose, the incidence of colic was suppressed and the amount of putrefactive compounds in their faeces decreased (Wolter 1999). An experiment conducted on 40 horses receiving increasing levels of oligofructose or lactulose in their diet confirmed the decrease of the incidence of colic and showed a proportional effect to the quantity of supplemented prebiotics (Orafti, personal communication).

Recent data showed that oligofructose can induce laminitis in bovine (Thoefner, Pollitt et al. 2004) and in horses (French and Pollitt 2004). This information should be taken cautiously. Oligofructose as many other carbohydrates (starch for example) is able to provoke digestive disorders when fed at significant large amount because it floods in the cecum or colon. The inductive dose that was fed to the animals was indeed 100 times higher than the doses currently recommended for prebiotic effect (10kg/day *versus* 0.1kg/day).

Microbial additives shall be harmless and unforeseen adverse effects can be occurring. A case in point is a recent study of a probiotic (*Lactobacillus pentosus* WE7) for prevention of diarrhea in neonatal foals (Weese and Rousseau 2005). Foals supplemented with this probiotic actually had a higher incidence of diarrhea, plus other clinical abnormalities such as anorexia, depression, weakness and colic. They required more veterinary examination and treatment than control foals, indicating that this specific 'pro'biotic was actually a pathogen. Adverse effects of probiotic supplementation may be more likely to occur in foals than adult horses, both because of the ability to deliver high per-kilogram doses to the small foals and because of their less developed intestinal microflora.

Saccharomyces boulardii has been shown to be effective in humans, but its effect in horses is unclear. Many investigations, conducted principally in hamsters, rats and mice, have shown the effectiveness of *Saccharomyces cerevisiae* (or *S. boulardii*) for the prevention of antibiotic associated diarrhea and the treatment of recurrent *Clostridium difficile* diarrhea. These beneficial effects would be of particular interest in horses.

In equine practice, regarding the different data obtained in humans or in other animal species, it is probable that some prebiotics and/or probiotics could be useful for treating or preventing certain diseases. However, extrapolations shall not be done in horses and more research has to be conducted to identify these specific products and diseases.

Effects on the GI microflora

Prebiotic compounds are not digestible feed ingredients by the host's digestive enzymes but are fermentable substrate by specific microflora of the digestive tract. They selectively promote the numbers and metabolic activity of lactic acid bacteria, and especially *Lactobacillus* spp. and *Bifidobacterium* spp., to the detriment of potential pathogenic bacteria such as *Clostridium* spp. Prebiotics that have been proven to positively interact with digestive microflora in other species must be specifically tested in horses to determine whether the same effects occur. At this point, few studies have been conducted about the action of prebiotics on the equine GI microflora.

Oligofructose appeared to be degraded to some extent by the microflora in the upper tract (Drogoul and Julliand 2005, report prepared for Orafti; (Respondek, Goachet et al. 2005). Therefore, major differences appeared in the stomach when oligofructose were supplemented: total bacteria as well as lactate-producers (*Streptococcus*) and lactate-utilising bacteria counts

increased but there was no change in lactate concentration as demonstrated in pigs and in rats (Tsukahara, Iwasaki et al. 2003). The pH was higher when oligofructose was supplemented, which may contribute to diminish the risk of gastric ulcers (Nadeau, Andrews et al. 2000). Inulin and oligofructose supplemented at 2% in an experimental pelleted concentrate appeared within less than three hours and remained during a few hours post-prandial. They were probably completely degraded by the cecal microflora as they could not be detected in the colonic content (Drogoul and Julliand 2005, report prepared for Orafiti). However, some changes were reported in the right ventral colon as well as in the cecum: VFA concentrations were lower six hours post-prandial when inulin or oligofructose was supplemented at 1% of the concentrate pellets (Drogoul and Julliand 2005, report prepared for Orafiti). This is in agreement with significantly higher pH values in the right ventral colon of horses fed fructooligosaccharides (Respondek, Goachet et al. 2005). The total anaerobes counts decreased and the streptococci concentration tended to decrease whereas the lactate-utilizers tended to increase (Respondek, Goachet et al. 2005). Opposite changes were reported in the feces due to the supplementation of oligofructose in the diets (Pellegrini, Miliani et al. 1999; Berg, Fu et al. 2005): counts of *E. coli* decreased; pH decreased whereas lactate and SCFA (acetate, propionate and butyrate) concentrations increased (Berg, Fu et al. 2005).

The addition of *Aspergillus oryzae* resulted in little change at the current recommended levels but improved *in vitro* fibrolytic activity at 10 times (McDaniel, Martin et al. 1993).

Total anaerobic and proteolytic bacterial concentrations increased in the cecum with a daily supplementation of 10^{10} spores of *Bacillus cereus*, strain BCIP5832, during 5 weeks (de Vaux and Julliand 1992).

Intestinal colonization of different strains of *Lactobacilli* have been tested recently: *Lactobacillus rhamnosus* strain GG, supplemented for 5 days at doses of 10^9 , 10^{10} or 5.10^{10} cfu. $50 \text{ kg}^{-1} \text{ bodyweight} \cdot \text{day}^{-1}$, showed a sporadic and poor colonization (Weese, Anderson et al. 2003). *L. pensosus* strain WE7, was recovered from the faeces after an oral administration. This strain was inhibitory *in vitro* against *Escherichia coli*, *Salmonella zooepidemicus*, *Clostridium difficile* and mildly against *Clostridium perfringens* (Weese, Anderson et al. 2004).

While competitive exclusion may play a role in probiotic therapy, it is unlikely to account for the wide array of beneficial effects that have been reported in other species. Alternative proposed mechanisms include antimicrobial factor production, immunoregulatory effects, anti-inflammatory effects, anticarcinogenic effects and direct effects on the intestinal mucosa.

Cells of *Saccharomyces cerevisiae* (SC) supplemented to horses supplemented daily were counted in the cecum and to a lesser extent in the right ventral colon (Moore and Newman 1994; Medina 2003). Recent data reported the kinetics of SC cells counts in the right ventral colon and the feces of horses supplemented at 0.1 or 0.01% (depending on the cell concentration) of a concentrate pelleted feed (Gobert, personal communication). These observations confirm that yeast can reach but not survive in the hindgut. Supplementing SC to horses may allow some of them to better tolerate high starch diets without developing digestive disorders. It was shown indeed that supplementing SC, especially to cereal-rich diets, enhanced the overall pH values and reduced the post-prandial decrease in the cecum and the colon (Moore and Newman 1994; Medina 2003) as well as the lactate concentration and the ratio lactate-utilizing to lactate-producing bacteria (Medina 2003). The molar percentage of acetate increases in the cecum and the colon with SC supplementation, in correlation with

an enhancement of cellulolytic counts (Moore and Newman 1994) or of the fibrolytic activity (Medina 2003).

In equine practice, remember that most zootechnical and clinical effects of pre - and probiotics are based on the impact of these nutritional supplements on the GI microflora. Thus a current use of pre - and probiotics require more studies to better understand the direct effects of these supplements on the GI microflora in the horse.

Furthermore, through their action, both pre - and probiotics probably contribute to the animal welfare. Prebiotic compounds better balance microbial fermentation into various short chain fatty acids, which probably improves intestinal barrier function. As for probiotics, they potentially prevent the development of pathogens during their passage through the intestinal tract.

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