Ruminant Session

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The Feed Industry from a European Perspective: Feed Additives from Non-antibiotic Origin in Growing and Lactating Cattle

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Take-Home Message

The use of antimicrobial agents as growth promoters in animal production has been a relatively common practice since early 50's. With time, there was (and still is) a growing concern that the rate of antibiotic–resistant bacteria would be greater than the rate at which new effective antibiotics are developed. Based on the evidence collected and pressure from public opinion and human health organizations, the EU passed a total ban on antibiotics as growth promoters on January 1st, 2006. The consequences of the ban were: 1) an initial decrease in animal performance (that has later being partially improved), 2) an initial increased use of antibiotics for therapeutic purposes in animal husbandry, 3) the appearance of a wide range of compounds that claim to be alternatives to antimicrobials, 4) a change in production systems and nutrition, and 5) an improved public perception about animal production. The consequences on animal performance were most drastic in piglets than in other species. But, overall public perception of animal production improved greatly. Since then, a vast array of alternative additives has been proposed, all with variable degrees of success. Finding replacements for feed antibiotics will likely involve the use of multiple products in the diet as well as management and nutrition changes. Among the many additives proposed as alternative to antibiotics organic acids and pre- and probiotics are showing the greatest promise.

Introduction

The growth promoter effect of antibiotics was discovered in the 1940s, when it was observed that animals fed dried mycelia of *Streptomyces aureofaciens* containing chlortetracycline residues improved growth performance. The mechanism of action of antibiotics as growth promoters is related to interactions with intestinal microbial population (Niewold, 2007). Early work (Coates et al., 1995) showed that administration of antibiotic growth promoters to germ-free animals had no effects. An additional mechanism that has been suggested is the improvement of nutrient digestibility through a reduction of gut wall and villus lamina propria (Anderson et al., 1999), although most of their effects are attributed to interactions with the gut microbiota (Dibner and Richards, 2005).

Addition of chloratetracycline, sulfamethazine, virginamycin, and tilmicosin to treat enteritis, coccidiosis, and bovine respiratory disease in the ration of cattle arriving at the feedlot was also reported to improve growth rate, dry matter intake, and feed conversion while decreasing the risk of bovine respiratory disease and incidence of liver abscesses (Galyean et al., 1995; Salinas-Chavira et al., 2009). Whether this is a direct effect of antibiotics on performance or a result of improved health status of the animals is unknown, but there is consistent evidence that bovine respiratory disease has a direct negative effect on growth both short- (Bach et al., 2011; Stanton et al., 2012) and long- (Bach, 2011) term.

Furthermore, coccidiostats, such as ionophores, were commonly used (and approved) for poultry in the 1940's. It was noted that cattle grazing these pastures grew more rapidly than cattle grazing pastures fertilized with manure from chickens that were not fed monensin (Brodway et al., 2014). As a result, ionophores (i.e., monensin) were directly incorporated into cattle rations in the early 1970's; a practice that continues today. Also, in the US, rations for feedlot cattle are typically supplemented with monensin and tylosin. The mode of action of ionophores consists of direct inhibition of Gram positive bacteria such as non-desired rumen bacteria as lactic acid bacteria and hyperammonia producing bacteria. Ionophores have been reported to reduce liver abscesses by inhibiting epithelial keratinization caused by rumen lactic acidosis and subsequent *Fusobacterium necrophorum* infections (Lechtenberg et al., 1998; Nagaraja and Chengappa, 1998), and have also been reported to increase growth performance through a variety of modifications of the ruminal microbial population (Callaway et al., 2003). Furthermore, ionophores have been described to increase energy and protein utilization in the rumen, and to improving production efficiency of dairy animals (Sprott et al., 1988; Duffield et al., 2008a,b,c).

In dairy production systems, young calves fed milk replacer still receive antibiotics on a routine and continual basis. A US study reported that 63% of dairy calves were fed milk replacer, and that in 1990 to 1991, nearly 60% of milk replacers fed to dairy calves less than 3 weeks of age were medicated (Heinrichs et al., 1995). Antibiotic use was even greater from 3 weeks to weaning, as 71% of milk replacers contained medication (Heinrichs et al., 1995). However, usage was reduced to 56% in 2001 (USDA, 2002), and this figure seems to have stabilized around 58% since then (USDA, 2010). Berge et al. (2005) concluded that removal of antibiotics from milk replacers may have a significant negative impact on calf health in the absence of adequate passive transfer; thus if antibiotics should be removed, adequate colostrum feeding practices and a clean and dry environment for calves is mandatory.

However, the inclusion of antibiotics (including monensin) in animal feeds is receiving increased criticism because of the potential for antibiotic residues and resistant strains of bacteria (Russell and Mantovani, 2002; Oliver et al., 2011). For example avoparcin, a glycopeptide antibiotic not used in humans but included in some feeds as a growth enhancer, has been known to be associated with emergence of avoparcin-resistant strains, which are cross-tolerant to vancomycin, a glycopeptide antibiotic used in humans (Marshall and Levy, 2011).

Antibiotic-resistant bacteria represent a potential risk for humans and for this reason, the World Health Organization (1997) and the Economic and Social Committee of the European Union (1998) concluded that the use of antimicrobials in animal feed was a public health issue. Sweden was the first EU country that discontinued the use of selected antibiotics as growth promoters in 1986, and Denmark withdrew avoparcin followed by the rest of the European Union in 1995. In 1999, the growth-promoting use of bacitracin, spiramycin, tylosin, and virginiamycin had ceased in Europe even though the EU Council stated that evidence of actual risk to human health was not forthcoming (EU Council, 1998).

Therefore, recent efforts have been made to identify alternatives to antibiotics that can increase production efficiency of livestock. This article will review the potential problems related to feed antibiotics to ruminants, the consequences of the EU ban in animal production, and the potential advantages of alternative additives for ruminant production.

The origin of the "resistance"

Two conditions are needed for antibiotic resistance to first develop in bacteria. First, the organism must come into contact with the antibiotic. Then, resistance against the agent must

develop, along with a mechanism to transfer the resistance to next generations or to other bacteria. Each antibiotic operates at a specific site of the bacteria. For example, some target the cell walls (i.e., bacitracin, and penicillin), whereas others target cell membranes (i.e., ionophores), cell protein synthesis (i.e., aminoglycosides, chloramphenicol, and tetracycline), RNA (i.e., rifamycins), DNA (i.e., nalidixic acid and quinolones) (Levy, 1998). Thus, when resistant organisms arise, their resistance is specific to a particular antibiotic or antibiotics that share the mode of action (Khachatourians, 1998). Four basic mechanisms of resistance have been documented: 1) development of mechanisms that prevent antimicrobial access to the site of action by increasing efflux or decreasing influx through the cell membrane; 2) development of enzymes that degrade or alter the antimicrobial agent; 3) alteration of the site of antimicrobial action, rendering the drug ineffective; and 4) development of site-of-action bypass mechanisms (Low, 2001). Genes encoding for these resistance can be chromosomal or extra-chromosomal. The extrachromosomal elements (plasmids and transposons) are small pieces of circular DNA and some can move from one bacterium to another irrespective whether or not antibiotics are present (Marshall et al., 1990). Unusual transfer of antibiotic-resistant DNA sequences between bacterial species and between different ecological niches (i.e., between humans and ruminants) have been documented (Khachatourians, 1998). However, most antibiotics used for treating infections are synthesized by microorganisms isolated from the environment, which suggests that genes for antibiotic resistance must also have emerged in non-clinical habitats (Martínez, 2008). For example, multidrug resistance efflux pumps are present in all organisms and can exist in large numbers within a single microorganism (Lubelski et al., 2007). Thus, it must be acknowledged that previously unrecognized antibiotic resistance genes that may emerge in the future already exist in many, as yet ignored, environmental organisms (Martínez et al., 2007), and thus the feed antibiotics ban will not totally solve the bottom-line thread, although it may greatly contribute to minimize the risk as it has been shown that antibiotics in animal feed may facilitate phage-mediated gene transfer and thus promoting dissemination of antibiotic resistance (Allen et al., 2011).

The consequences of the ban

The complete ban of feed antibiotics in the EU has led to five main consequences: 1) an initial decrease in animal performance (that was later partially improved), 2) an initial increased use of non-feed antibiotics for therapeutic purposes in animal husbandry, 3) the appearance of a wide range of compounds that claim to be alternatives to antimicrobials, 4) a change in production systems and nutrition schemes, and 5) an improved public perception about animal production. The latter is of great importance as, after all, animal production should satisfy the nutritional needs of the consumer using acceptable means by the society.

Early reports regarding the effects of the voluntary ban of feed-antibiotics in Denmark indicated that there was no effect on broiler productivity or longevity (Emborg et al., 2002), but feed efficiency decreased a 0.94% from November 1995 to May 1999 (from 56.2 to 55.7%), with feed efficiency reaching 54.6% immediately after the ban to a nadir lesser than 54.3% in late 1999 (Emborg et al., 2002). Feed efficiency progressively improved after nutritional, management, and therapeutic measures were implemented. For instance, the use of salinomycin in 1996 was 4,500 kg and 11,213 kg in 2002 (DANMAP, 2005), which probably reflects attempts by producers to use this drug to control necrotic enteritis since the feed antibiotic ban in Denmark in 1999 (Dibner and Richards, 2005). In swine, the effects of the ban were more evident, with a decline in average daily gain in piglets from 422 g in 1995 to 415 g/d in 2001 and an increase from 2.7 to 3.5% in mortality over the same period (Callesen, 2003). But, there has been no major effect of the feed antibiotic termination on productivity or feed efficiency in finishers (Middelbo, 2003). On the other hand, the ban seemed to have a beneficial impact on the

isolation of resistant bacteria from human stools (Figure 1), and this encouraged other EU countries to implement a similar ban.
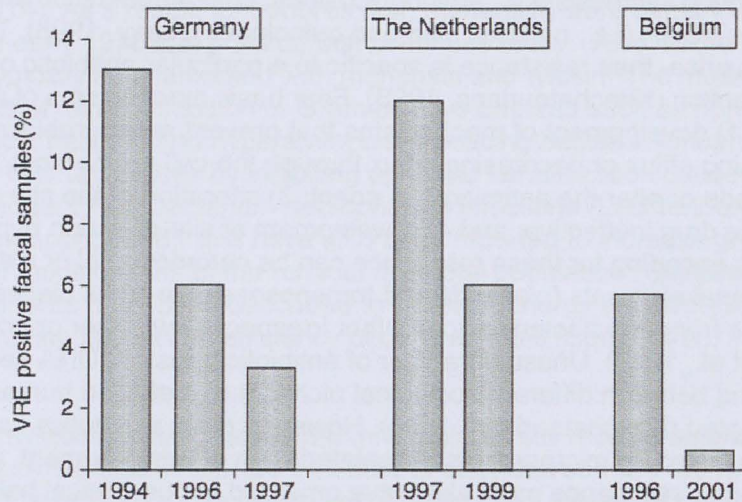

Figure 1. Vancomycin-resistant *Enterococcus* (VRE) in stool culture samples from healthy humans in the community (The Netherlands and Germany) and in hospitalized patients (Belgium) following the European Union prohibition of the glycopeptide avoparcin as a growth promoter. Adapted from Wegener, 2003.

As a result of the ban and a focus on disease prevention and conscious use of antimicrobials implemented in Sweden, the total use of antibiotics in farm animals decreased by approximately 55% between 1986 and 1999, and a relatively low prevalence of antimicrobial resistance has been maintained since then Wierup (2001). In Denmark, as a consequence of the implementation of the voluntary antibiotic ban, the total use of antimicrobials decreased from about 200 tons in 1994 to 94 in 2001, but there was an increase of about 5% in the antibiotics used for therapeutic treatments of farm animal (DANMAP, 2005). Similar trends have been observed in other EU countries.

There are several reports indicating that the growth-promoter ban has driven an increase in infections and therefore an increase in the use of therapeutic antibiotics for livestock animals in Europe although the ban reduced the overall antibiotic use in animals (Figure 2). This increase in therapeutic use of antibiotics elicited a concern: whether an increase in antimicrobial resistance among *Salmonella typhimurium* and *Campylobacter jejuni* human isolates to tetracycline and other antimicrobials observed in Denmark in 2001 following the voluntary ban (Hayes and Jensen, 2003) was due to the increase in therapeutic use of antibiotics in farm animals (including tetracycline, aminoglycosides, macrolides and lincosamides which are also used human medicine). This concern was raised because antibiotics that were banned were active against Gram-negative bacteria and could not have created resistance in Gram-positive bacteria such as *Salmonella* or *Campylobacter* (Hayes and Jensen, 2003), but due to health problems that were encountered following the ban, many antimicrobials in use before the ban were replaced with tetracycline (which is active against Gram Positive bacteria). Tetracycline use in Denmark went from 12,100 kg in 1998 to 27,900 kg in 2001, and Denmark experienced problems with tetracycline resistance in humans. Similarly, the use of these compounds to treat infections caused by *Enterobacter* and *Campylobacter* in the Netherlands led to increases in the number of fluoroquinolone-resistant strains, which appeared in humans who consumed poultry (Endtz, 1991). The prevalence of enrofloxacin-resistant strains of *Campylobacter* in poultry and

humans increased from 0% to 14% and from 0% to 11% respectively, and Koenaard et al. (1995) reported a greater prevalence of quinolone-resistant *Campylobacter* isolates from sewage plants receiving effluents from poultry processing plants. However, as time has passed since the ban, production systems have been progressively adapted with implementing reductions in stocking density and improvements in hygiene as well as nutritional changes that have allowed to reduce pathology and recover a large proportion of the performance lost when the feed antibiotics were initially removed.
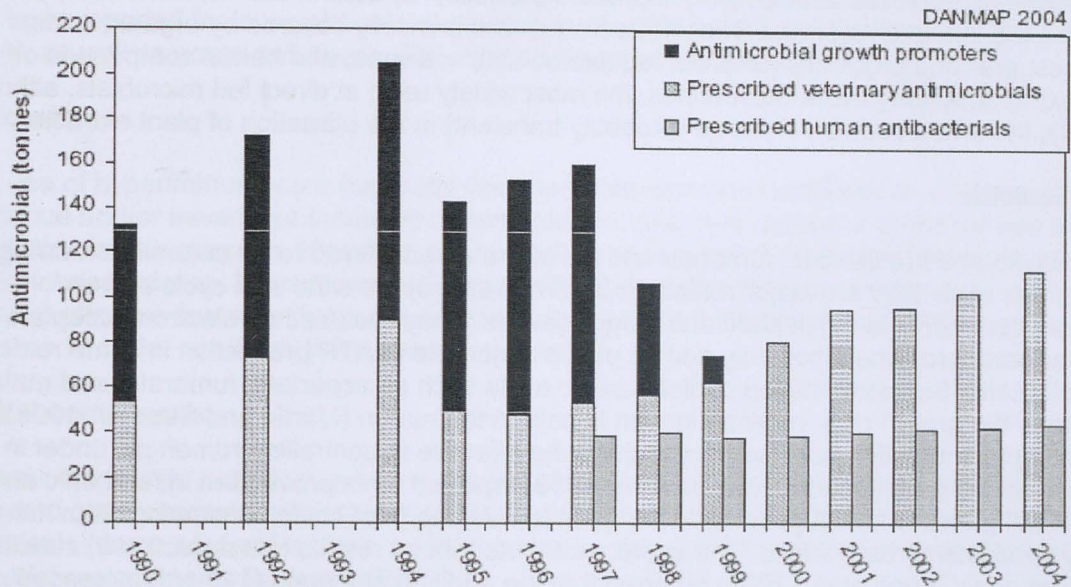

Figure 2. Evolution of antibiotic use in farm animals in Denmark
(Adapted from DANMAP, 2004).

A European study conducted in 6 countries reported a significant decrease in the prevalence of *E. faecium* strains resistant to spiramycin and tylosin or to virginiamycin when comparing a period before and after the EU ban of these antibiotics (Bywater et al., 2005). However, there was no change in the resistance to glycopeptides (as expected as avoparcin use had already been discontinued earlier). Similarly, streptogramin resistance rates fell from 40 to 60% in the late 1990s to 16% in pigs and 13% in chickens in Denmark (DANMAP, 2005). The WHO (2003) concluded that the ban was successful in reducing the resistance gene pool, despite the fact that several macrolides, previously used both for growth promotion and for the prevention and treatment of infections, continued to be used legitimately for disease control, and their use for therapy increased after the ban (DANMAP, 2005). Thus, the main problem with the increased use of antibiotic for therapeutic use is that some of these antibiotics (contrarily to the feed antibiotics) belong to the same families to those used in humans (and in occasions is the exact same antibiotic) and this practice might have posed a greater threat than that posed by feed antibiotics. For this reason, now in Europe there is an increasing concern, and likely an upcoming regulation, to limit, restrict, and define when and what type of antibiotic should be administered to livestock for therapeutic purposes. The concern originated mainly in The Netherlands, and was driven by the usage of antibiotics in livestock that are of critical importance for public health such as the fluoroquinolones and the 3rd and 4th generation cephalosporins.

Lastly, for ruminants, a major drawback of the ban of feed antibiotics has been a drastic increase in the incidence of coccidia in young stock (both dairy and beef) due to the absence of

monensin in the grower feed. There have been several attempts to effectively decrease the incidence of this disease, but results have been either deceiving or not cost-effective.

The alternatives

Since the complete ban of feed antibiotics in 2006, in addition to changes in nutrition and management practices, a myriad of feed additives has been proposed as alternative. The proposed feed additives attempt to 1) improve digestibility, 2) control the intestinal flora, and 3) enhance the immune response. These three areas are basically covered by organic acids, enzymes, pre- and probiotics (or direct fed microbials), vaccines, and herbal compounds or plant extracts. Among these compounds, the most widely used at direct fed microbials, although recently, there has been an increase (probably transient) in the utilization of plant extracts.

Organic acids

Dicarboxylic acids (aspartate, fumarate and malate) are considered to be naturally occurring substances since they are major metabolic intermediates of the citric acid cycle and are therefore commonly found in plant and animal tissues. They are used as electron acceptors in the succinate–propionate pathway and so play a major role in ATP production in some rumen microbial cells. Supplementation of dicarboxylic acids such as aspartate, fumarate, and malate, stimulates the growth of *S. ruminantium* on lactate in the rumen (Martin and Streeter, 1995). Malate supplementation has been proposed to be effective in controlling rumen pH under in vitro conditions (Martin, 1998). Martin et al. (1999) reported an improvement in feed efficiency and animal growth when supplementing malate to finishing beef cattle consuming diets rich in rapidly available carbohydrates. The authors attributed these results to a reduction in subclinical rumen acidosis. Kung et al. (1982) reported that the addition of malate (140 g/d) increased milk persistency and VFA production. However, this level of supplementation is cost-prohibitive. Devant et al. (2007) used a lower and more practical dose (although still relatively expensive) of malate, and reported no differences in ruminal pH when supplementing dairy cattle with 80 g of malate per day during transition and early lactation. A potential more cost-effective alternative to the inclusion of malate in cattle rations could be the use of alfalfa or other forage varieties high in malate (Callaway et al., 2000).

Enzymes

The rumen ecosystem harbors about 10^{10} microorganisms/ml, and is composed of several hundred species (Edwards et al., 2004; Larue et al., 2005). These species possess a vast array of enzymes acting concertedly to degrade the plant substrates present in feeds. Some exogenous enzymes are resistant to ruminal degradation (Hristov et al., 1998) and may offer potential for improving diet digestibility and animal performance. The addition of exogenous amylase to the ration is one method of enhancing ruminal digestibility of both starch and nonstarch carbohydrates (Tricarico et al., 2008; DiLorenzo et al., 2010).

Most studies conducted on exogenous enzymes have targeted dairy cattle, mainly due to the high passage rate of the digesta in lactating cows. Klingerman et al. (2009) reported that addition of exogenous amylase to a 25% starch diet increased milk yield and Gencoglu et al. (2010) reported that the addition of exogenous amylase to a 21% starch diet increased apparent total-tract digestibilities of organic matter and fiber, decreased feed intake, and increased fat-, solids- and energy-corrected milk feed conversions; but Ferraretto et al. (2011) reported no advantages when adding amylase to 22% starch diets. Results of a more recent study indicated that amylase increased nutrient digestibility, but this did not translate into improved milk performance (McCarthy et al., 2013). Thus, the responses to exogenous enzymes in dairy cattle

seem variable and more research is needed to identify the diets where the advantage of these compounds can be most evident. Nevertheless, in a review of 20 experiments consisting of 41 treatments, enzyme feed additives increased milk production an average of 1.2 kg/day (Beauchemin et al., 2003). This value is close to the 1.1 kg/day average milk production increase reported by Kung (1998) when summarizing 32 lactation studies supplementing direct-fed microbials.

Although there are fewer data, a positive response to supplementation of exogenous enzymes has been reported for in weight gain of growing beef cattle has (Beauchemin et al., 2001 and 2004), as well as goats and sheep (Titi, 2003; Titi and Lubbadeh, 2004).

Vaccines

The use of hyperimmune sera (antibody vaccines) has also been proposed to minimize the incidence and/or severity of subacute rumen acidosis, and thus reduce or avoid the use of antibiotics for this purpose. However, antibodies are proteins and may not resist digestion or ruminant degradation. Nevertheless, Gill et al. (2000) reported that vaccination against *S. bovis* seemed effective in preventing rumen acidosis in sheep, but there is no evidence that it is effective in cattle, nor it has been commercialized.

Herbal compounds

Herbal compounds including essential oils (volatile oils obtained from plants, possessing the odor and other characteristic properties of the plant), herbs (plants with medical properties) and botanicals (drugs made of a plant, as from roots, leaves, etc...) have also been proposed as potential replacements for feed antibiotics.

Flavonoids, have been proposed as an alternative to decrease pathogen shedding into the food supply (Holiman et al., 1996; Mandalari et al., 2007). Flavonoids have an antioxidant activity (Pietta, 2000) and have been reported to decrease the viability of pathogenic bacteria such as *E. coli* and *Salmonella*, as well as other microorganisms such as *Candida albicans* and *Sacchromyces* species (Friedman, 2007).

Tannins (phenolic compounds that bind to proteins and other large molecules) have been used to slow the degradation rate of proteins in the rumen (Aerts et al., 1999; Barry and McNabb, 1999) and perhaps improve performance. Saponins are glycosides found in many plants that can alter the cell wall of Gram-positive bacteria such as *Ruminococcus flavefaciens* and *Ruminocossus coccus albus* without compromising the cellulolytic activity of Gram-negative such as *Fibrobacter succinogenes* (Wina et al., 2006).

Several plant extracts, especially essential oils, have biologically active molecules with antimicrobial activities that protect plants from pathogens and herbivores. Essential oils are lipophilic, and thus interact with the cell membrane of bacteria, which accounts for their toxicity and antimicrobial effects, particularly against Gram positive bacteria. Several plant extracts, including garlic oil, cinnamaldehyde (from cinnamon), and eugenol (from cloves), have been reported to influence rumen fermentation in vitro (Calsamiglia et al., 2007), but evidence of their effectiveness in vivo is currently lacking, and doses used *in vitro* could not realistic be extrapolated to *in vivo* conditions (Gauthier, 2005). A few investigations have been conducted to determine the effect of various plant extracts on production performance of dairy cows. For example, feeding a blend of thymol, eugenol, vanillin, guaiacol, and limonene increased feed intake and fact-corrected milk of lactating dairy cows fed a moderate dose (600 mg/d; Kung et al., 2008); whereas at greater doses, it had no effect on intake or production although it

increased rumen pH either at 750 mg/d (Benchaar et al., 2007) or at 2 g/d (Benchaar et al., 2006). However, in another study, in which dairy cows were fed an intermediate dose (1.2 g/d) of the same blend of plant extracts the authors reported a decrease in feed intake and no effect on milk production (Tassoul and Shaver, 2009). Supplementing a blend of eugenol, geranyl acetate, and coriander led to a decrease in feed intake but an increase in milk fat yield, indicating a potential shift in energy usage for milk fat synthesis (Santos et al., 2010). Supplementation of lactating dairy cows with a blend of cinnamaldehyde, thymol, and orange peel at 640 mg/d increased both the fat and protein contents of milk, but had no effect on feed intake or milk production (Spanghero et al., 2009), and a blend of cinnamaldehyde and eugenol fed at about 500 mg/d had no effect on intake or performance (Tager and Krause, 2011; Tekippe et al., 2013) an when fed at an extremely high dose (10 g/d) impaired rumen fermentation (Tager and Krause, 2011).

A widespread use of plant extracts in the future as performance enhancers for ruminants seems to be limited due to 1) a high variability (and difficulty to assess it) in quality and quantity of the active compound in the feed (this variation is due in part to the fact that accumulation of secondary metabolites is greatly affected by environmental conditions and cultivar variety), 2) some of the bioactive compounds have antioxidant activity, and this property can be lost during storage (Jouany and Morgavi, 2007), and 3) inclusion rates depend on the chemical composition of the plant preparation (Cordell, 2000). In addition, essential oils are "generally recognized as safe" but they must be used cautiously because they can be toxic (allergens) and their odor/taste may result in feed refusal (Lis-Balchin, 2003). Furthermore, they are part of the defense mechanism used by plants against pathogens and herbivores and little information is available on the transfer of these substances into edible animal products and their possible toxicity for consumers (Jouany and Morgavi, 2007) .Currently, the EU is allowing the use of essential oils and other plant extracts as flavor enhancers and as such, do not require to be evaluated by European Food Safety Authority (EFSA) as effective in fostering improvements in animal production. This 'back door' has been used by the industry to position plant extracts in the market.

Lastly, it is rather surprising that the EU banned monensin but currently allows the use compounds with potential antimicrobial activity such as essential oils with exact modes of actions (in some cases) to those of monensin. For example, a described mode of action for the essential oil carvacrol is the potassium efflux (Ultee et al., 1999), the same action mode as that of monensin (currently banned in the EU).

Direct-fed microbial

Ionophores, such as monensin, have been (and in many areas of world still are) included in ruminant diets to mitigate the negative consequences of acute rumen acidosis (Nagaraja et al., 1987; Mutsvangwa et al., 2002). Similarly, the non-ionophore antibiotic tylosin is commonly used (also in some parts of the world) to reduce lactic acid production in the rumen (Nagaraja et al., 1987) and control the growth of *Fusobacterium necrophorum* (Lechtenberg et al., 1998), thought to be partly responsible for the occurrence of liver abscesses. In the EU, the control of acute rumen acidosis after the antibiotic ban has mainly relied on direct fed microbials (both bacteria and yeast).

Bacteria

Both, lactic acid producing bacteria and lactic acid utilizers have been proposed as potential alternatives to control rumen acidosis. For instance, supplementation of *Enterococcus faecium* have been reported to increase ruminal pH (Ghorbani et al., 2002; Nocek and Kautz, 2006) and

maintenance of an active lactate-utilizing population, such as *Megasphaera elsdenii*, in the rumen (Jouany and Morgavi, 2007; Nocek and Kautz, 2006). However, Raeth et al. (2007) supplemented lactating cows with *Lactobacillus acidophilus* and *Propionibacteria freudenreichii* and reported no effects on cow performance, diet digestibility, or rumen fermentation.

In addition, it appears that the supplementation of *Lactobacillus* species may exert further benefits as a probiotic in the lower digestive tract (Brown and Nagaraja, 2009). On the other hand, lactic acid utilizers, such as *Megasphaera elsdenii*, have been reported to be effective in decreasing the accumulation of lactic acid in *in vitro* systems and avoiding the pH decline in both *in vitro* and *in vivo* (Hino et al., 1994; Wiryawan and Brooker, 1995).

Lastly, there are several bacteria that produce bacteriocins. Bacteriocins are proteins that inhibit the growth of other bacteria and are synthesized by bacteria occupying the same environment (Jack et al., 1995). The presence of bacteriocins in the rumen has been reported by several authors (Wells et al., 1997; Russell and Mantovani, 2002), and some of the them, such as those produced by *Lactococcus* have the ability to inhibit the growth of *E. coli* O157:H7 (Peterson et al., 2007; Rozema et al., 2009). However, further research is needed to assess when these bacteriocins and produced and how to control them.

Live yeast, yeast fermentation products, and yeast cell wall products

In spite of the increasing number of studies on bacterial probiotics, by far the most commonly used probiotics in adult ruminants are based on yeast preparations of *Aspergillus orizae* and (or) *Saccharomyces cerevisiae*, with the latter, being the most commonly used.

Yeasts are aerobic and cannot survive for long in an anaerobic environment such as the rumen. For this reason, they must be supplied continuously in feeds to reach the minimum effective concentration, set at 10^5 colony-forming units per gram of rumen content (Jouany and Morgavi, 2007). Live yeast, in particular *Saccharomyces cerevisiae* has been proposed as a potential alternative to monensin. Yeasts seem particularly useful in high-producing ruminants whose digestive microbial balance can be altered by high-dietary energy input (Chaucherays-Durand et al., 2008). Some live yeasts have been described to stimulate rumen bacterial growth through the provision of growth factors (Callaway and Martin, 1997) as well as increased nutrient digestion (Wohlt et al., 1991). Direct comparisons between monensin and yeast are scarce. A recent study (Swyers et al., 2014) compared the use of monensin and *Saccharomyces cerevisiae* fermentation product in yearling beef steers and reported that that carcasses from yeast-supplemented animals were lighter, although a greater proportion of them were graded as USDA choice than animals that were supplemented with monensin. In dairy cattle, supplementation of live yeast has been reported to increase milk production and dry matter intake (Jouany, 2006; Sniffen et al., 2004; Stella et al., 2007).

As live yeast can survive and remain metabolically active in the gut, they can exert probiotic effects by interacting with the autochthonous microbial species responsible for feed digestion (Chaucherays-Durand et al., 2008). Up to now, the most consistent positive effects of live yeast have been reported on rumen microbial activity in young ruminants, stabilization of rumen pH, and prevention of rumen acidosis, as well as stimulation of growth and activity of fiber-degrading bacteria (Chaucherays-Durand et al., 2008). The ability of life yeast to control lactic acid concentrations in the rumen has been reported in rumen-cannulated dairy cows (Williams et al, 1991) and using *in vitro* incubations with mixed ruminal microorganisms (Lila et al., 2004). This effect could be explained by the fact that one strain of *S. cerevisiae* has been shown to outnumber *S. bovis* in the utilization of sugars, consequently limiting the amount of lactate produced in the rumen (Chaucheyras et al., 1996). Similar to observations made with

Enterococcus strains and *A. oryzae*, some species of yeast have been shown to stimulate the growth of a specific lactic acid-utilizing bacteria in the rumen such as *Selenomonas ruminantium* (Nisbet and Martin, 1991; Rossi et al., 2004), by supplying different growth factors including amino acids, peptides, vitamins, and organic acids. Bach et al. (2007) reported that supplementation of live dry yeast (*S. cerevisiae*) increased the average rumen pH and average maximum pH by 0.5 units, and average minimum pH by 0.3 units in loose-housed lactating cows. Furthermore, the authors described a significant change in the eating behavior of the animals. Cows supplemented with live yeast had a shorter inter-meal interval (3.32 h) than unsupplemented cows (4.32 h). It has been suggested that this change in feeding behavior could also be responsible for changes in rumen pH. A similar study by Thrune et al. (2009) from the University of Minnesota reported an increase in average rumen pH of 0.2 units when comparing live yeast-supplemented cows with unsupplemented cows kept in tie-stalls.

Aspergillus oryzae is the most common fungus used as a probiotic. This fungus has been reported to improve fiber digestion in the rumen (Judkins and Stobart, 1988; Fondevilla et al., 1990), but with no effects on rumen volatile fatty acid or ammonia N concentrations (Gomez-Alarcon et al., 1990). Some strains of *A. oryzae* have been reported to stimulate the growth of *M. elsdenii* (Waldrip and Martin, 1993) and *Selenomonas ruminantium* (Nisbet and Martin, 1993), which both actively metabolize lactic acid into propionic acid, thus reducing the risk and severity of acidosis. However, positive effects of *A. oryzae* on modulating rumen pH have not been found in the literature.

Yeast cell wall products are another type of feed supplement that have been fed to livestock as a means to improve animal performance and gut health by impeding some bacteria to attach to their target intestinal cells. Yeast cell wall components have been reported to function as immunomodulators that activate macrophages, neutrophils, and other immunocompetent cells (Onderdonk et al., 1992; Eicher et al., 2006;). Yeast cell wall products have also been shown to improve metabolism in heifers during an endotoxin immune challenge (Burdick Sanchez et al., 2014).

Fructo oligosaccharide inclusion in human diets appears to result in fecal bulking and selective stimulation of bifidabacterium growth in the colon (Van Loo et al., 1999). Mannan oligosaccharides contain cell wall fragments obtained from *Saccharomyces cerevisiae*. Mannan oligosaccharides have improved performance in nursery pigs (Dvorak and Jacques, 1998) and weight gain and feed intake in dairy calves (Dvorak and Jacques, 1997). Because many Gram negative bacteria attach to the intestinal epithelium using mannose-specific fimbriae (Ofek et al., 1977), mannan oligosaccharides provide competitive binding sites for these intestinal pathogens. Feed intake has been reported to increase in mannan oligosaccharide-fed calves compared with antibiotic-fed calves, but this difference did not result in growth differences during the experimental period (Heinrichs et al., 2003). Although mannan oligosaccharides have been described to alter lymphocyte response *in vitro* (Muchmore et al., 1990), their effects on animal immune system are not well established. The supplementation of mannan oligosaccharides in the milk replacer stimulated starter intake after weaning, but this supplementation did not have a determining effect on reducing bacteria counts or *Cryptosporidium* spp. presence in calf feces, nor in rate of growth (Terré et al., 2007).

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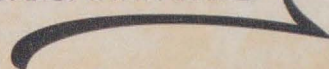World Health Organization. 2003. Impacts of antimicrobial growth promoter termination in Denmark. Foulum, Denmark: WHO.