Review

Alternatives to antibiotics for farm animals

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Abstract

Nowadays, there is an increasing concern for the public health about the consequences from the long and increased use of antibiotics in livestock production. The use of antibiotics in animal feed as growth promoters has been completely banned by the European Union since 2006, based on their possible negative effects on human and animal health. The removal of growth promoters has led to animal performance problems and a rise in the incidence of certain animal diseases. Thus, there is an urgent need to find alternatives to antibiotics, especially in EU. Due to the modern consumers' concern about the potential development of antibiotic-resistant bacteria as well as at the same time the need to prevent economic losses of the farmers, alternatives to antibiotics has been developed to prevent the health problems and to improve the growth performance in farm animals. Owing to the full ban of antibiotic use in EU there is an urgent need to find alternatives to in-feed antibiotics. New strategies and commercial products must be developed to improve animal health and performance, based on their safety, efficacy and cost-effectiveness. The aim of this study is to summarize the beneficial effects of currently used alternatives to in-feed antibiotics, i.e. probiotics, prebiotics, organic acids, phytogenic compounds and zeolites on health and growth performance in farm animals (swine, poultry and ruminants).

Keywords: Animal, Antibiotic, Acid, Probiotics, Prebiotics, Phytogenics, Zeolites

Introduction

For several decades, the use of sub-therapeutic levels of antibiotics in animal feeds has been a common practice in many countries in order to improve growth performance and prevent from the adverse effects of pathogenic and non-pathogenic enteric micro-organisms. However, there are increasing concerns for the public health about the consequences from the use of antibiotics in livestock [1]. The risk of developing cross-resistance and multiple-antibiotic resistance in pathogenic bacteria both in human and farm animals, has been strongly linked to the therapeutic, metaphylactic or prophylactic uses of antibiotics in human and veterinary medicine, as well as growth promoters in animal feed [2].

The use of antibiotics as growth promoters has been complete banned by the European Union (EU) since 2006 (EC Regulation No. 1831/20031), based on their possible negative consequences for animal health and food safety [3, 4]. This ban has led to animal performance problems and a rise in the incidence of certain diseases [5, 6]. Thus, there is an urgent need to develop alternatives to antibiotics, especially in EU. As a consequence of the public health concerns and the demand of the farmers to prevent the economic losses, non-antibiotic additives have been developed for prophylactic use against pathogens or as growth promoters.

The aim of this review is to present the beneficial effects of some currently used alternatives to in-feed antibiotics, i.e. probiotics, prebiotics, organic acids, phytogenics and zeolites on health as well as on growth performance of farm animals.

Alternatives to Antibiotics

Organic acids – acidifiers

Organic acids are considered to be any organic carboxylic acid of the general structure R-COOH. They are widely distributed in nature as normal constituents of plants or animal tissues and also formed through microbial fermentation of carbohydrates, mainly in the large intestine. They are sometimes found as their sodium, potassium or calcium salts. Most organic acids with specific antimicrobial activity are short-chain acids (C1–C7, SCFA) and they have a pK_a – between 3 and 5.

The most common organic acids (also called acidifiers) that are used in farm animal feed are formic, acetic, propionic, butyric, lactic, sorbic, fumaric, tartaric, citric, benzoic and malic [7]. According to their effects, they can be categorized into two groups: (a) the first group (lactic, fumaric, citric) is characterized by indirectly reduction of bacterial populations by decreasing pH in the stomach, and (b) the second group (formic, acetic, propionic and sorbic) is characterized by a direct effect of lower pH in the gastrointestinal (GI) on the cell wall of Gram-negative bacteria [8–10].

The mechanisms of their action include reduction of gastric pH or buffering capacity of diets, increase of proteolytic enzymes activity and nutrient digestibility, improvement of pancreatic secretions, stimulation of digestive enzymes activity, balancing the microbial population and promotion of beneficial bacterial growth, reduction of pathogens survival through the stomach and direct killing of bacteria [11–15].

Their effects depend on several factors as: type and pK_a of acid, inclusion rate of supplemented acids, composition of diets and their acid–base or buffering capacity, level of intraluminal production of acids in GI tract by inhabiting microflora, feed palatability, intrinsic acid activity, receptors for bacterial colonization on the epithelial villi, maternal immunity by vaccinations, hygiene and welfare standards, age of animals [11, 16–18].

Swine

Many studies proved benefits from the use of dietary acidifiers in swine, including positive effects on growth performance as well as on prevention and control of diseases. Their antimicrobial effects depend on their concentration and pH [19]. For example, lactic acid is more effective in reducing gastric pH and coliforms [20–22], whereas other acids (e.g. formic, propionic) have broader antimicrobial activities and they can be effective against bacteria (e.g. coliforms, clostridia and Salmonella), fungi and yeast [11, 22–24]. Several studies have reported reduction of coliforms burden along the GI tract, decrease of piglet scouring or mortality as well as effective control of post-weaning diarrhoea and oedema disease in piglets [21, 25–27].

Acidifiers have received much attention in pig production owing to their beneficial effects on growth performance by improving digestive processes through several mechanisms [12, 18, 25, 27–33]. They can improve gut health by promoting the beneficial bacterial growth, while inhibiting growth of pathogens (through reduction of pH and buffering capacity of diets). A reduced buffering capacity of diets containing organic acids is also expected to slow down the proliferation and/or colonization of undesirable microbes, e.g. *Escherichia coli*, clostridia, *Salmonella* spp. in the gastroileal region [11, 20, 24, 26, 33–36]. Acidifiers can also stimulate pancreatic secretions [37], which increase the digestibility, absorption and retention of protein and amino acids [38, 39] and minerals (e.g. Ca, P, Mg and Zn) [29, 32] in the diet.

Poultry

Organic acids are formed through microbial fermentation of carbohydrates predominantly in the caeca of poultry [40]. The mechanism of their action probably reflects their antibacterial nature, such as decreasing the pH of drinking water and reducing the buffering capacity of the feed with subsequent effect on the physiology of the crop and proventriculus [41, 42].

Acidifiers reported to have beneficial effects on poultry performance or health. For example, some (e.g. butyric acid) also decrease the incidence of subclinical necrotic enteritis caused by *Clostridium perfringens*, which is highly relevant for the poultry industry [43]. Butyric acid has also anti-inflammatory effects [44] and has been shown to strengthen the gut mucosal barrier by increasing production of antimicrobial peptides in mucous and by stimulating the expression of tight junction proteins [45–48]. Moreover, there is some evidence of increased growth of the GI mucosa in the presence of organic acids, particularly fatty acids such as butyric acid. Indeed, butyric acid has been shown to be an important energy source for gut epithelial cells and to stimulate epithelial cell proliferation and differentiation [49].

Ruminants

Organic acids establish their antimicrobial effect in the intestines by suppressing fungal activity and maintaining an acidic environment [7]. The main organic acids of interest in ruminants are malate, fumarate and aspartate. Acidifiers are reported to improve rumen fermentation, like ionophor antibiotics and maintain the rumen pH even after consuming carbohydrate-rich feeds through, which increased growth performance, are achieved [50].

In addition to buffering effect in rumen, acidifiers might increase energy-efficiency and digestibility of crude protein, Ca and P by lowering methane production and decreasing the numbers of harmful bacteria attached to the intestinal wall [51].

Limited *in vivo* research has been conducted to evaluate the effects of organic acids on ruminant performance. Malate supplementation reported to increase nitrogen retention in sheep and steers and improve growth performance in bull calves [50, 52, 53]. Additionally, acidifiers such as malate and fumarate can improve milk production [54–57].

Phytogenics

The interest in phytogenic feed additives has considerably increased during the past years. Phytogenic feed additives are commonly defined as plant-derived compounds incorporated into farm animals' diets, such as herbs, spices and essential oils [58, 59]. They have beneficial effects on farm animals, including improvement of growth parameters through amelioration of feed properties, promotion of the animals' production performance, and improving the quality of food derived from those animals [58].

Swine

The mode of their action as feed additives is still not fully understood. However, many studies reported antimicrobial, antioxidative and growth-promoting effects [58–60]. The potential mechanisms of their action include: (a) antimicrobial effects: Oregano and thyme are among those which have received a great deal of interest [58, 60–62]; (b) antioxidative effects: phytogenic feed additives derived from plants high in terpenes (e.g. rosemary, oregano and thyme) have anti-oxidative properties, mainly due to their phenolic terpenes [58, 63–65]; (c) growth-promoting effects (increased feed intake, improved gut function and dietary palatability): their stimulatory effect on feed intake is probably due to the improvement in the dietary palatability of resulting from the enhanced flavour and odour, especially with the use of essential oils [65, 66].

Recent studies indicated stabilizing effects (essential oils and oleoresins) on the ecosystem and the activity of GI microbial flora of swine [51, 66–68] associated with a decrease in microbial activity of the GI gut. Improvement in gut function is mainly attributed to the possible stimulatory effect of phytogenic substances on digestive secretions, such as digestive enzymes, bile and mucus [69]. Based on Greek experience, the use of phytogenics can have significant antimicrobial activity against Gram-negative bacteria (mainly *E. coli*), antioxidative action, enhance dietary palatability, improve the gut functions and promote growth performance and carcass quality of pigs [60, 70–73].

Poultry

The phytogenic additives are a proven dietary supplement for poultry, containing a proprietary blend of plant extracts (essential oils, bitter substances, pungent substances and saponins). Some of these compounds stimulate appetite (e.g. menthol from peppermint), provide antioxidant protection (e.g. cinnamaldehyde from cinnamon) or suppress microbial growth (carvacrol from oregano). Because of possible 'synergy' between constituents, it remains unclear which components of etheric oil products may stimulate the endogenous digestive enzymes, act as an antioxidant, antimicrobial agent or immunomodulator. *In vitro* studies indicated antimicrobial effects with respective minimum inhibitory concentration (MIC)-values and spectrum of activity [74–76]. The antimicrobial activity is rather weak for ginger and pepper, medium for cumin (p-cymene), coriander (lialol), oregano (carvacrol), rosemary (cineol), sage (cineol) and thyme (thymol) and strong for clove (eugenol), mustard (allylisothiocyanate), cinnamon (cinnamaldehyde) and garlic (allicin) [77].

The essential oils stimulate the intestinal endogenous enzymes. Essential oils from oregano are showing the greatest potential as an alternative to antibiotic growth promoters. Oregano contains phenolic compounds (e.g. carvacrol) that have antimicrobial activity [78]. Oregano essential oils can modify the gut microflora and reduce microbial load by suppressing bacteria proliferation. There are some claims that oregano oil can replace anticoccidial compounds, not because they inactivate coccidia, but because they increase the turnover of the gut lining and prevent coccidial attack by maintaining a more healthy population of gut cells [79]. This mode of action would increase the animal's maintenance energy requirement because enterocyte turnover is a major proportion of the basal metabolic rate.

Bitter substances are found in herbs and stimulate the secretion of gastric juices. The pungent substances are found in plants such as paprika, garlic and onion, and are purported to function by increasing blood circulation, leading to faster detoxification of the whole metabolism. Saponins enhance the permeability of the gut wall and reduce ammonia. Flavonoids are plant polyphenols with anti-inflammatory effects and they also help to maintain the health of small blood vessels and connective tissue.

Ruminants

Tannins: Two categories of tannins exist: condensed tannins or proanthocyanidins and hydrolysable tannins. As they can form chemical complexes with proteins, they slow dietary protein ruminal breakdown, enhance small intestine amino-acid bioavailability and reduce ruminal NH_3 production and nitrogen (N) excretion in urine. The prevention of ruminal protein degradation improves the nutritional status and reduces the amount of N released environmentally [80–82].

Ruminal and intestinal feed digestion is modified by tannins' antimicrobial effects, which lower bacterial concentrations in the rumen and decrease the bacterial proteins' amounts flowing to the intestine [83]. Condensed tannins increase the microbial protein synthesis efficiency by redirecting a higher proportion of fermented nutrients to microbial mass synthesis at the expense of volatile fatty acids (VFA) production [80, 84–88].

The methane produced per unit of digestible dry matter is reduced (20–30%) when ruminants consume forages rich in tannins (*Lotus pedunculatus*, *Lotus corniculatus* etc.) [89–91]. Severity of bloat legumes is lowered when condensed tannins are present, since gas formation and microbial protein degradation decrease [92–94].

Tannins can substitute chemical anthelmintics in controlling gastrointestinal parasitic nematodes [95–97]. Tannins have also exhibited a direct antiparasitic activity, while stimulating host resistance as a result of an increase in intestinal protein supply [98].

Saponins: Lucerne and soybeans are the main examples of saponin-rich plants used in ruminant diets [99]. They have hypocholesterolaemic, anticoagulant, anticarcinogenic, hepatoprotective, hypoglycaemic, immunomodulatory, neuroprotective, anti-inflammatory and anti-oxidant activities [82, 100]. Their action depends on dosage rate and rumen pH [101–103].

Dietary supplementation of ruminant diets with saponins supposedly improves growth, feed efficiency and health [104]. Their effects based on their effect on ruminal microbes, result in a decrease in fed proteins' degradability in the rumen in conjunction with an increase of microbial protein synthesis, which in combination increase the intestinal flow of amino acids [105]. Eventually, saponin administration improves nitrogen digestion, since less NH_3 is produced in the rumen and less urea is eliminated in urine [85, 102]. Ruminal NH_3 concentration and methane production are also significantly decreased [88, 102, 106–109]. Saponins can alter the cell wall structure of Gram-positive bacteria and because of their strong inhibiting effect on *Saccharomyces cerevisiae* [110], it is strongly recommended that they are not used associated with yeast-based probiotics. They can also exhibit antimicrobial action by increasing bacterial membranes' porosity [110, 111]. Additionally, bacterial growth inhibition may be caused by complication of essential minerals and steroids with saponins, consequently limiting their bioavailability for bacterial metabolism [112]. Moreover, the antiprotozoal property of saponins could be exploited in the treatment of protozoal infections in ruminants [113, 114]. Finally, saponins (e.g. extracted from *Sesbania sesban* leaves or lucerne roots) can reduce protozoal numbers [81, 115–117].

Essential oils: Because of their lipophilic nature, essential oils interact with the cell membrane of bacteria, thus acquiring their toxic and antimicrobial effects, especially against Gram-positive bacteria. The external capsule of Gram-negative bacteria can protect them against essential oils [118, 119], but some are small enough to enter the inner membrane and damage it. They can also cause coagulation of cytoplasmic material [120] and impair fungal, protozoal and viral growth [119, 121–125].

The effectiveness of commercial blends of essential oils depends on the protein source [126, 127]. Garlic oil, cinnamaldehyde, eugenol, carvacrol and thymol, are more active on rumen fermentations, such as depress NH_3 and methane production and improve propionate production at the expense of acetate. Most depress NH_3 and methane production and improve propionate production at the expense of acetate [128–133]. Garlic oil could have the potential to inhibit methanogens without affecting other rumen micro-organisms [134]. Optimal doses of essential oils are difficult to assess, because of vast differences in chemical composition between preparations. Differences in efficacy of the same essential oil mixture may be explained by their ability to adsorb on the surface of some dietary ingredients and more specifically affect the microbes attached to them [135]. Thus, ration composition may modulate the response of rumen microbes to essential oil addition. The simplest and most economically efficient method of delivering bioactive plant secondary metabolites to farm animals would be to feed them with a fresh or dried plant.

Probiotics

Probiotic comprised of individual species or mixtures of lactic acid bacteria, yeasts or their end products. Probiotics for use in farm animals are typically divided into the following categories: (1) live cultures of yeast or bacteria, (2) heat-treated (or otherwise inactivated) cultures of yeast or bacteria or (3) fermentation end products from incubation of yeast or bacteria. The mechanisms of their action include (a) competition between yeast or bacteria of probiotics and pathogenic micro-organisms in the intestinal mucosa [136–138], (b) nutrient availability [138, 139] and (c) total inhibition of pathogen growth by production of organic acids and antibiotic-like compounds [138–141].

The most commonly used probiotic bacterial strains are *Bifidobacterium (B. bifidum*, *B. pseudolongum)*, *Lactobacillus* (e.g. *L. acidophilus*, *L. casei*, *L. rhamnosus*), *Bacillus* (e.g. *B. subtilis*, *B. cereus*, *B. toyoi*, *B. licheniformis*), *Lactococcus* (e.g. *L. lactis*), *Enterococcus* (e.g. *E. faecium*), *Streptococcus* (e.g. *S. thermophilus*), *Pediococcus* and *Saccharomyces* (e.g. *S. cerevisiae*). Many studies demonstrated their beneficial effects on health and growth performance of farm animals [60, 142–144]. In particularly, probiotics have positive effects on: (a) the digestive process by increasing the activity of microbial probiotic enzymes and the digestibility of food [145], (b) immunity by stimulating the immune system and the regeneration of intestinal mucosa (e.g. macrophages and natural killers cells, increase of immunoglobulin production, regulate anti- and pro-inflammatory cytokine production) [145–148].

The effects by the use of probiotics in animals' feed depends on the combination of selected bacteria, doses in feed, and on their interactions with pharmaceuticals, feed composition, storage conditions and feed technology [149–151].

Swine

Probiotics in swine can inhibit pathogenic micro-organisms, improve the intestinal microflora and stimulate immune by modulating intestinal microflora and/or lowering the pH value in the small intestine and producing organic acids and antibacterial substances [140, 141, 144, 152–155]. For example, members of the genus *Bacillus* support natural intestinal microflora, compete with undesirable micro-organisms, and reduce the numbers of Enterococci, Bacteroides and coliforms [149, 152, 156–159] the bacteria *E. faecium* were found to be able to prevent the K88 positive ETEC strain from adhering to the intestinal mucous membrane of piglets [158, 160] or regulating intestinal microbial balance by increasing the activity of microbial digestive enzymes [138].

Beneficial effects of probiotics have reported in the health status and growth performance in newborn and weaned piglets [151–155, 158, 161]. In the post-weaning period, probiotics could be used for the prevention of post-weaning diarrhoea caused by enterotoxigenic *E. coli* strains [138, 140, 141, 145, 162].

Poultry

The most well-known group of probiotics are lactic acid bacteria. It has been shown that lactic acid produced *in vitro* by lactic acid bacteria is used by the strictly anaerobic butyrate producing bacteria of clostridial clusters IV and XIV for the production of large concentrations of butyric acid [163]. This mechanism is called cross-feeding and is a further reason why lactic acid bacteria administrations have beneficially performance. The intestinal microbiota have a specific multifactorial 'barrier' impact, such as (1) induction of anatomical and physiological changes in the intestinal cell wall structure, (2) immunological modifications in the gut and (3) enhancement of the bird's resistance to enteropathogenic bacteria, such as *C. perfringens* [164–167]. Depending on the probiotic strain, the mode of action probably involves production of specific metabolites (short organic fatty acids, H_2O_2, intermediary metabolites with antimicrobial activity), interaction with receptor sites, stimulation of the immune system and some others [168, 169].

Ruminants

Probiotics are generally recommended in ruminants' nutrition whenever a risk of rumen dysfunction exists, in order to improve anaerobiosis, stabilize pH and supply nutrients to microbes in their microenvironment. Probiotics are recommended in young ruminants [170, 171] to prevent diarrhoea caused by enterotoxigenic bacteria in the gut and also during weaning period to enhance the rate at which rumen flora and fauna become established. *L. acidophilus* alone or in combination with other lactobacilli has been shown to reduce scouring and increase growth rate of calves in some trials [172, 173].

Bacterial probiotics have been predominantly promoted to prevent ruminal acidosis. Lactic acid-producing and lactic acid-utilizing bacteria are used, sometimes combined, to reduce the negative impact of rapid fermentation of high-starch feeds in the rumen. Lactate utilizers such as *Megasphaera elsenii* or *Selenomonas ruminantium* have been reported to prevent lactate accumulation and alleviate the drop in ruminal pH when animals are fed high-starch or high-sugar diets [174]. *Propionibacteria* are also used for their lactate-utilizing activity and high production of propionate. The rationale for the utilization of lactate producers, such as *Lactobacillus* and *Enterococcus* sp., is that by maintaining a low and constant level of lactic acid, they sustain an active population of lactate utilizers that in turn will prevent lactate accumulation and ruminal pH drop [175]. The most commonly used probiotics in adult ruminants, however, are those based on yeast preparations of *Aspergillus orizae* and (or) *S. cerevisiae*. Live yeasts are mainly used because they act as regulators for rumen pH and prevent its drop when diets rich in fermentable carbohydrates are fed [176]. They may also exert an effect in the post-rumen digestive compartments as about 17–34% of administered yeasts remain alive during transit along the gut of ruminants [177].

The addition of probiotics in lambs, calves or dairy cattle diets seems to have beneficial effects on their performance (e.g. average daily gain, feed intake) [178–181] and especially in milk production (e.g. higher production numerically, increased milk fat and protein) [182–186].

Prebiotics

Prebiotics are dietary short-chain carbohydrates (oligosaccharides). They have beneficial effects on health and growth performance in farm animals, stimulating the growth and/or activity of one or more of beneficial bacteria. The non-digestibility of prebiotics ensures that they can reach the colon and act as an energy source for bacteria, unlike normal sugars, which get digested directly by the host [187]. As a result, the composition and/or the activity of the microbiota are altered, leading to secondary effects such as increased gas production and a drop in pH. Prebiotics can also prevent the adhesion of pathogens to the mucosa, by competing with its sugar receptors and several studies have shown that supplementing feed with various oligosaccharides have led to reduced susceptibility to *Salmonella* and *E. coli* colonization [188–191].

The most common non-digestible oligosaccharides (NDO), which are used as prebiotics in farm animals, are the following: mannanoligosaccharides (MOS), galactooligosaccharides, fructooligosaccharides (FOS), soybeanoligosaccharides, isomaltooligosaccharides, xylo-oligosaccharides, lactulose and inulin [192–195].

Swine
MOS have beneficial effects on the intestinal microflora by modifying the microbial gut ecology and preventing the colonization of bacterial pathogens (e.g. stimulate the growth of non-pathogenic bacteria such as *B. longum*, *L. casei*, *L. acidophillus* or *L. delbrückei* and suppress the growth of pathogenic bacteria such as *E. coli*, *Salmonella typhimurium*, *Clostridium botulinum* and *C. sporogenes*) [193, 196, 197].

The adding of indigestible NDO to the animal feed based on either fructose or mannose sugars derived from yeast-cell wall can be used to attract pathogenic bacteria to attach to these dietary particles rather than the intestinal cells. Bifidogenic effects of galactooligosaccharides, FOS and soybeanoligosaccharides have been reported in many *in vitro* and *in vivo* studies [162, 194, 198].

Lactulose is formed by lactose isomerization and it cannot be absorbed from the small intestine. Therefore, it passes down to the large intestine, resulting in lactic and/or acetic acid production by the resident microflora [187]. Therefore, it stimulates the growth and/or activity of indigenous intestinal microflora, especially of the genera *Bifidobacterium* and *Lactobacillus*, and reduces the activity of proteolytic bacteria. The dosage of 1% lactulose is usually adding in swine diets for the prevention or control of enteric infections [141, 187].

Inulin is present in many vegetables (e.g. onion, garlic, asparagus and banana) [190]. Its supplementation has positive effects on SCFA production, sufficient height of intestinal villi, stimulation of natural microflora and improvement of performance parameters [199].

Poultry
Two of the most commonly studied prebiotic oligosaccharides in poultry are FOS and MOS. The supplementation of poultry feed with MOS resulted in an improvement in intestinal morphology and intestinal enzyme activity, yet the growth performance of the broilers was not up to the level of including an antibiotic growth promoter to the feed [200]. Mannose, the main component of MOS, is a unique sugar because many enteric bacteria have receptors that bind to it. These receptors, called Type 1 fimbriae, are involved in attachment of the bacteria to the cells of the host. Attachment is critical for the bacterium to be able to cause disease in the host. Chickens likely have receptors for Type1 fimbriae in their small intestine [201]. MOS functions as a competitive binding site; the bacteria bind to it and are carried out of the gut rather than binding to the intestine. In a study that supports this theory, it was found that supplementing the drinking water of broilers with 2.5% mannose reduced *S. typhimurium* colonization of the intestines [202].

Studies with adding FOS in poultry diets reported significant reduction in *Salmonella* carriage in the ceca [203] significant improvements on growth performance [204]. Results obtained from synthetic materials suggest some benefits using inulin and FOS that act as substrates for 'desired' micro-organisms, for example Bifidobacteria [188, 205–207], whereas MOS have receptor properties for fimbriae of *E. coli* (sensitive to mannose) and *Salmonella* spp., which leads to elimination of these bacteria with the digesta flow instead of binding a mucosal receptor [150, 208–210].

Oligosaccharide β-glucans of yeast cell wall origin are thought to stimulate performance because of their immunomodulatory effects. Recent reviews elaborate on the action of glucans on immune stimulation [211, 212].

Zeolites

Zeolites are crystalline, hydrated aluminosilicates of alkali and alkaline earth cations, with infinite structures which are three-dimensional. Based on their unique properties, zeolites (especially clinoptilolite), have been used as feed additives in order to ameliorate mycotoxicosis and improve animals' performance. Recently, clinoptilolite has been approved as feed additive in EU at the highest inclusion rate of 2% of dry matter. Its effectiveness on mycotoxins' binding as well as the increased interest for organic products that favours the use of feed additives, which have no residuals on animal products, are expected to increase the use of clinoptilolite as feed additive.

Swine
The addition of clinoptilolite in swine rations has positive effects on growth performance of growing and fattening pigs [212] and carcass characteristics [213, 214]. It is believed that enhanced swine performance by clinoptilolite results from its direct binding effects to some harmful by-products of the intestinal flora (e.g. ammonium ions, *p*-cresol) [215].

Additionally, the use of clinoptilolite as feed additive during pregnancy has beneficial effects on reproductive traits of sows, increasing litter size and body weight (BW) at birth and weaning [212, 216, 217] and reducing the interval between weaning and mating [212].

Poultry
In laying hens, the administration of clinoptilolite improves feed conversion rate [218], increases the number of eggs laid [218, 219] and improves their quality characteristics [218–220]. In broilers, clinoptilolite accelerates their growing rate by increasing feed consumption [221, 222] and feed conversion rate [222, 223] and improves carcass quality by lowering fat percentage [223, 224].

In ostriches, it has been reported that clinoptilolite affects the total bacterial counts of the eggshells. Dedousi *et al.* [225] observed that its use as nest material in ostriches reduces the total bacterial counts of eggshells compared to river sand. This finding was attributed to the fact that clinoptilolite adsorbed and immobilized the bacteria from nest environment, resulting in a net reduction of their number. As a consequence, the number of free micro-organisms able to infect the eggs laid in nests with clinoptilolite, was less than those in the nests with other materials.

Ruminants

Clinoptilolite acts as a regulatory factor when added to acidic or basic aqueous solutions [226]. Recent studies proved that the administration of clinoptilolite in dairy cows (200 g daily or 1.4% dry matter) resulted in significantly higher pH values [227–229]. Dschaak *et al.* [229] further observed that the pH values of cows fed clinoptilolite were comparable to those obtained from cows consuming equal amounts of sodium bicarbonate, concluding that clinoptilolite can cost-effectively replace sodium bicarbonate as ruminal buffer.

Recent studies have shown that the use of clinoptilolite as a feed additive can prevent ETEC diarrhoea by increasing intestinal immunoglobulin absorption in newborn calves. The administration of clinoptilolite (5 g/kg BW) along with colostrum can increase the degree of absorption of colostral IgG, as well as blood serum concentrations of IgG in dairy calves [230, 231]. Moreover, the use of 25 ml of clinoptilolite suspension (20% in distilled water) in the colostrum can increase the apparent intestinal absorption of colostral IgG and blood serum concentration of IgG in newborn calves [232]. Recently, Pourliotis *et al.* [233] proved that the administration of clinoptilolite with colostrum initially, and milk afterwards (1 g/kg BW and 2 g/kg BW/day during the first 10 days) is associated with: (a) significantly higher antibody titres against *E. coli* in blood serum of calves and the incidence of ETEC diarrhoea was significantly lower in calves that were receiving clinoptilolite, (b) increase of the intestinal absorption of immunoglobulins either by increasing the pinocytotic activity of intestinal epithelial cells or by retarding the intestinal passage rate, (c) increase the time that immunoglobulins are available to the specific receptors of the epithelial cells, (d) bind some degradation products of the colostral proteins in the intestine that have negative effect on the intestinal epithelial cells, such as NH_3. The shorter duration of ETEC diarrhoea incidences in experimental calves was further attributed to the alteration of metabolic acidosis, through clinoptilolite effects on osmotic pressure in the intestinal lumen and to the absorption by clinoptilolite of bile acids (endogenic cause of diarrhoea) and glucose (high content in intestinal fluid acts as an irritant factor).

The administration of clinoptilolite in sheep can be beneficial for the prevention of certain parasitic infections. In ewes, its dietary inclusion (2.5% of the concentrates) during the transition period can reduce *Eimeria* oocyst output [234]. In addition, the use of clinoptilolite supplementation in lambs can decrease their total worm burden and faecal egg counts per capita and reduce the establishment of GI nematodes [235]. Moreover, the use of clinoptilolite as feed additive in dairy goats seems to improve their energy status. Its dietary inclusion (2.5% of the concentrates) during the transition period can reduce the blood serum concentration of β-hydroxybutirate and increase the BW of triplets and quadruplets kids at birth [236]. Moreover, the administration throughout lactation can increase the milk fat content and reduce the somatic cell counts in milk [236].

Conclusions

Modern animal production is trapped between concerns on risks for public health and an increasing demand for animal origin products. The increased concern about the potential for developing antibiotic resistant strains of bacteria within the food chain, especially after the ban of non-therapeutic antibiotics in animal feed in EU leads to an increased development and research on alternatives to antibiotics for use as feed additives in livestock.

Alternatives to antibiotics could be important tools for veterinary practice in case they can improve growth performance of farm animals at levels comparable to antibiotics. For this reason, new strategies and commercial products must be developed, based on their cost-effectiveness as well as on their efficacy to minimize or eliminate the pathogen load in the livestock and food chain. The future studies should be focused on strategies to develop commercial products not only in agreement with the modern consumer demands for more environmental friendly animal production (e.g. organic farming), but also supporting the farmers needs for higher livestock production. In conclusion, useful tools for farmers and veterinarians' to improve the animal health and performance could be products such as probiotics, prebiotics, organic acids or zeolites.

References

1. Phillips I. Assessing the evidence that antibiotic growth promoters influence human infections. Journal of Hospital Infections 1999;43:173–8.
2. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. Journal of Nutrition 1995;125:1401–412.
3. EFSA -European Food Safety Authority. The community summary report on trends and sources of zoonoses and zoonotic agents in the European Union in 2007. EFSA Journal 2009;223:1–320.
4. Fernando U, Biswas D, Allan B, Wilson P, Potter AA. Influence of *Campylobacter jejuni fliA*, *RpoN* and *Flgk* genes on colonization of the chicken gut. International Journal of Food Microbiology 2007;118(2):194–200.
5. Wierup M. The Swedish experience of the 1986 ban of antimicrobial growth promoters, with special reference to animal health, disease prevention, productivity, and usage of antimicrobials. Microbial Drug Resistance 2001;7:183–90.

6. Dibner JJ, Richards JD. Antibiotic growth promoters in agriculture: history and mode of action. Poultry Science 2005;84:634–43.

7. Dibner J, Buttin P. Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism. Journal of Applied Poultry Research 2002;11:453–63.

8. Roth FX, Kirchgessner M, Eidelsburger U, Gedek B. Nutritive efficacy of *Bacillus cereus* as a probiotic in the fattening of calves. 1. Influence on fattening performance, carcass criteia and microbial activity in the small intestine. Agribiological Research-Zeitschrift fur Agrarbiologie Agrikulturchemie Okologie 1992;45:294–302.

9. Hansen LL, Larsen AE, Jensen BB, Hansen-Moller J. Short time effect of zinc bacitracin and heavy fouling with faeces plus urine on boar taint. Animal Science 1997;64:351–63.

10. Castro M. Use of additives on the feeding of monogastric animals. Cuban Journal of Agricultural Science 2005;39:439.

11. Partanen HK, Mroz Z. Organic acids for performance enhancement in pig diets. Nutrition Research Reviews 1999;12(1):117–45.

12. Partanen K. Organic acids – Their efficacy and modes of action in pigs. In: Piva A, Bach Knudsen KE, Lindberg JE, editors. Gut Environment of Pigs. Nottingham University Press, Nottingham, UK; 2001. p. 201–18.

13. Knarreborg A, Miquel N, Granli T, Jensen BB. Establishment and application of an *in vitro* methodology to study the effects of organic acids on coliform and lactic acid bacteria in the proximal part of the gastrointestinal tract of piglets. Animal Feed Science and Technology 2002;99(1–4):131–40.

14. Diebold G, Eidelsburger U. Acidification of diets as an alternative to antibiotic growth promoters. In: Barug D, de Jong J, Kies AK, Verstegen MWA, editors. Antimicrobial Growth Promoters. Wageningen Academic Publishers, The Netherlands; 2006. p. 311–27.

15. Tung CM, Pettigrew JE. Critical review of acidifiers. National Pork Board. 2006. Available from: URL: http://www.pork.org/Documents/PorkScience/.

16. Strauss G, Hayler R. Effects of organic acids on microorganisms. Kraftfutter 2001;4:147–51.

17. Decuypere JA, Dierick NA. The combined use of triacylglycerols containing medium-chain fatty acids and exogenous lipolytic enzymes as an alternative to in-feed antibiotics in piglets: concept, possibilities and limitation. An overview. Nutrition Research Reviews 2003;16:193–209.

18. Mroz Z. Organic acids as potential alternatives to antibiotic growth promoters for pigs. Advances in Pork Production 2005;16:169–82.

19. Chaveerach P, Keuzenkamp DA, Urlings HAP, Lipman JA, van Knapen F. *In vitro* study on the effect of organic acids on *Campylobacter jejuni/coli* populations in mixtures of water and feed. Poultry Science 2002;81(5):621–8.

20 Jensen BB, Mikkelsen LL, Canibe N, Høyberg O. *Salmonella* in slaughter pigs. Annual Report 2001 from the Danish Institute of Agricultural Sciences, Research Centre Foulum, Tjele, Denmark; 2001. p. 23.

21. Tsiloyiannis VK, Kyriakis SC, Vlemmas J, Sarris K. The effect of organic acids on the control of porcine post-weaning diarrhoea. Research in Veterinary Science 2001a;70(3):287–93.

22. Øverland M, Kjos NP, Borg M, Sørum H. Organic acids in diets for entire male pigs. Livestock Production Science 2007;109(1–30):170–3.

23. Bosi P, Sarli G, Casini L, De Filippi S, Trevisi P, Mazzoni M, *et al.* Effect of dietary addition of free or fat-protected calcium formate on growth, intestinal morphology and health of *E. coli* k88 challenged weaning pigs. Italian Journal of Animal Science 2005;4(2):452–4.

24. Creus E, Perez JF, Peralta B, Baucells F, Mateu E. Effect of acidified feed on the prevalence of *Salmonella* in market-age pigs. Zoonoses and Public Health 2007;54(8):314–9.

25. Piva A, Casadei G, Biagi G. An organic acid blend can modulate swine intestinal fermentation and reduce microbial proteolysis. Canadian Journal of Animal Science 2002;82(4):527–32.

26. Piva A, Grilli E. Role of benzoic, lactic and sorbic acid *in vitro* swine cecal fermentation. Veterinary Research Communications 2007;31(1):401–4.

27. Papatsiros VG, Tassis PD, Tzika ED, Papaioannou DS, Petridou E, Alexopoulos C, *et al.* Effect of benzoic acid and combination of benzoic acid with probiotic containing *Bacillus cereus* var. *Toyoi* in weaned pig nutrition. Polish Journal of Veterinary Sciences 2011;14(1):117–25.

28. Canibe N, Højberg O, Højsgaard S, Jensen BB. Feed physical form and formic acid addition to the feed affect the gastrointestinal ecology and growth performance of growing pigs. Journal of Animal Science 2005;83(6):1287–302.

29. Jongbloed AW, Mroz Z, van der Weij-Jongbloed R, Kemme PA. The effects of microbial phytase, organic acids and their interaction in diets for growing pigs. Livestock Production Science 2000;67:113–22.

30. Partanen K, Siljander-Rasi H, Alaviuhkola T, Suomi K, Fossi M. Performance of growing–finishing pigs fed medium- or high-fibre diets supplemented with avilamycin, formic acid or formic acid-sorbate blend. Livestock Production Science 2001;73(2–3):139–52.

31. Partanen K, Siljander-Rasi H, Pentikäinen J, Pelkonen S, Fossi M. Effects of weaning age and formic acid-based feed additives on pigs from weaning to slaughter. Archives of Animal Nutrition 2007;61(5):336–56.

32. Valencia Z. Phytase and acetic acid supplementation in the diet of early weaned piglets: effect on performance and apparent nutrient digestibility. Nutrition Research 2002;22(5):623–32.

33. Biagi G, Piva A, Hill T, Schneider DK, Crenshaw TD. Low buffering capacity diets with added organic acids as a substitute for antibiotics in diets for weaned pigs. In: Proceedings of the 9th International Symposium on Digestive Physiology in Pigs, May 14–17. University of Alberta, Department of Agriculture, Food and Nutritional Science, Edmonton, Banff, Alberta, Canada; 2003. p. 217–9.

34. Best P. Adding acids to swine diets. Feed Management 2000;51(5):19–22.

35. Naughton PJ, Jensen BB. A bioreactor system to study survival of Salmonella typhimurium in pig gut content. Berliner und Münchener Tierärztliche Wochenschrift 2001;114(1):1–4.

36. Tsiloyiannis VK, Kyriakis SC, Vlemmas J, Sarris K. The effect of organic acids on the control of post-weaning oedema disease of piglets. Research in Veterinary Science 2001b;70(3):281–5.

37. Harada E, Niiyama M, Syuto B. Comparison of pancreatic exocrine secretion via endogenous secretin by intestinal infusion of hydrochloric acid and monocarboxylic acid in anesthetized piglets. Japanese Journal of Physiology 1986;36(5):843–56.

38. Blank R, Mosenthin R, Sauer WC, Huang S. Effect of fumaric acid and dietary buffering capacity on ileal and fecal amino acid digestibilities in early weaned pigs. Journal of Animal Science 1999;77(11):2974–84.

39. Kemme PA, Jongbloed AW, Mroz Z, Kogut J, Beynen AC. Digestibility of nutrients in growing-finishing pig is affected by *Aspergillus niger* phytase phytate and lactic acid levels 1. Apparent ileal digestibility of amino acids. Livestock Production Science 1999;58(2):107–17.

40. Van Der Wielen PW, Biesterveld S, Notermans S, Hofstra H, Urlings BA, Van Knapen F. Role of volatile fatty acids in development of the cecal microflora in broiler chickens during growth. Applied and Environmental Microbiology 2000;71:2206–7.

41. Thompson JL, Hinton M. Antibacterial activity of formic and propionic acids in the diet of hens on *Salmonella* in the crop. British Poultry Science 1997;38:59–65.

42. Van Immerseel F, Russell JB, Flythe MD, Gantois I, Timbermont L, Pasmans F, *et al.* The use of organic acids to combat Salmonella in poultry: a mechanistic explanation of the efficacy. Avian Pathology 2006;35:182–8.

43. Timbermont L. A contribution to the pathogenesis and treatment of *Clostridium perfringens* associated necrotic enteritis in broilers [Ph.D. thesis]. Faculty of Veterinary Medicine, Ghent University, Belgium; 2009.

44. Hodin R. Maintaining gut homeostasis: the butyrate-NF-kappaB connection. Gastroenterology 2000;118:798–801.

45. Mariadason JM, Barkla DH, Gibson PR. Effect of short-chain fatty acids on paracellular permeability in Caco-2 intestinal epithelium model. American Journal of Physiology 1997;272:705–12.

46. Schauber J, Svanholm C, Termén S, Iffland K, Menzel T, Scheppach W, *et al*. Expression of the cathelicidin LL-37 is modulated by short-chain fatty acids in colonocytes: relevance of signalling pathways. Gut 2003;52:735–41.

47. Bordin M, D'Atri F, Guillemot L, Citi S. Histone deacetylase inhibitors up- regulate the expression of tight junction proteins. Molecular Cancer Research 2004;2:692–701.

48. Peng L, He Z, Chen W, Holzman IR, Lin J. Effects of butyrate on intestinal barrier function in a Caco-2 cell monolayer model of intestinal barrier. Pediatry Research 2007;61:37–41.

49. Dalmasso G, Nguyen HT, Yan Y, Charrier-Hisamuddin L, Sitaraman V, Merlin D. Butyrate transcriptionally enhances peptide transporter PepT1 expression and activity. PLoS ONE 2008;3:2476.

50. Martin SA, Streeter MN, Nisbet DJ, Hill GM, Williams SE. Effects of dl-malate on ruminal metabolism and performance of cattle fed a high concentrate diet. Journal of Animal Science 1999;77:969–75.

51. Castillo C, Benedito JL, Mendez J, Pereira V, Lopez-Alonso M, Miranda M, *et al*. Organic acids as a substitute for monensin in diets for beef cattle. Animal Feed Science and Technology 2004;115:101–16.

52. Stallcup OT. Influence of addition of DL malic acid to diets of lactating dairy cows. Journal of Dairy Science 1979;62(Suppl. 1):225–6.

53. Sanson DW, Stallcup OT. Growth response and serum constituents of Holstein bulls fed malic acid. Nutrition Reports International 1984;30:1261–7.

54. Kung L, Huber JT, Krummrey JD, Allison L, Cook RM. Influence of adding malic acid to dairy cattle rations on milk production, rumen volatile acids, digestibility and nitrogen utilization. Journal of Dairy Science 1982;65:1170–4.

55. Devan M, Bach A. Effect of malate supplementation on rumen fermentation and milk production in postpartum cows. Journal of Dairy Science 2004;87(Suppl. 1):47.

56. Sniffen CJ, Ballard CS, Carter MP, Cotanch KW, Dann HM, Grant RJ, *et al*. Effects of malic acid on microbial efficiency and metabolism in continuous culture of rumen contents and on performance of mid-lactation dairy cows. Animal Feed Science and Technology 2006;127:13–31.

57. Khampa S, Wanapat M, Wachirapakorn C, Nontaso N, Wattiaux M. Effect of levels of sodium dlmalate supplementation on ruminal fermentation efficiency in concentrates containing high levels of cassava chip in dairy steers. Asian-Australasian Journal of Animal Sciences 2006;3:368–75.

58. Windisch W, Schedle K, Plitzner C, Kroismayr A. Use of phytogenic products as feed additives for swine and poultry. Journal of Animal Science 2008;86(14):140–8.

59. Jacela JY, De Rouchey JM, Tokach MD, Goodband RD, Nelssen JL, Renter DG, *et al.* Feed additives for swine: fact sheets – prebiotics and probiotics, and phytogenics. Journal of Swine Health and Production 2010;18(3):132–6.

60. Papatsiros VG, Tzika ED, Papaioannou DS, Alexopoulos C, Tassis PD, Kyriakis SC, *et al.* Effect of *Origanum vulgaris* and *Allium sativum* extracts for the control of proliferative enteropathy in weaning pigs. Polish Journal of Veterinary Science 2009;12(3):407–14.

61. Hagmuller W, Jugl-Chizzola M, Zitterl-Eglseer K, Gabler C, Spergser J, Chizzola R, *et al.* The use of *Thymi herba* as feed additive (0.1%, 0.5%, 1.0%) in weanling piglets with assessment of the shedding of haemolysing *E. coli* and the detection of thymol in the blood plasma. Berliner und Munchener Tierarztliche Wochenschrift 2006;119:50–4.

62. Neill CR, Nelssen JL, Tokach MD, Goodband RD, DeRouchey JM, Dritz SS, *et al.* Effects of oregano oil on growth performance of nursery pigs. Journal of Swine Health and Production 2006;14:312–6.

63. Aeschbach R, Loliger J, Scott BC, Murcia A, Butler J, Halliwell B, *et al*. Antioxidant actions of thymol, carvacrol, 6-gingerol, zingerone and hydroxytyrosol. Food and Chemical Toxicology 1994;32:31–6.

64. Jimenez-Alvarez D, Giuffrida F, Golay PA, Cotting C, Lardeau A, Keely BJ. Antioxidant activity of oregano, parsley, and olive mill wastewaters in bulk oils and oil-in-water emulsions enriched in fish oil. Journal of Agricultural and Food Chemistry 2008;56:7151–9.

65. Frankic T, Voljc M, Salobir J, Rezar V. Use of herbs and spices and their extracts in animal nutrition. Acta Agriculturae slovenica 2009;94:95–102.

66. Kroismayr A, Sehm J, Pfaffl M, Plitzner C, Foissy H, EttleT, *et al*. Effects of essential oils or avilamycin on gut microbiology and blood parameters of weaned piglets.

Journal for Land Management, Food and Environment 2007;81:1–4

67. Manzanilla EG, Perez JF, Martin M, Kamel C, Baucells F, Gasa J. Effect of plant extracts and formic acid on the intestinal equilibrium of early-weaned pigs. Journal of Animal Science 2004;82(11):3210–8.

68. Namkung H, Li M, Gong J, Yu H, Cottrill M, de Lange CFM. Impact of feeding blends of organic acids and herbal extracts on growth performance, gut microbiota and digestive function in newly weaned pigs. Canadian Journal of Animal Science 2004;84:697–704.

69. Platel K, Srinivasan K. Digestive stimulant action of spices: a myth or reality? Indian Journal of Medical Research 2004;119:167–79.

70. Tzika ED, Papatsiros VG, Tassis PD, Koylialis D, Kritas S, Alexopoulos C, *et al.* Evaluation of the in feed use of larch sawdust in growing pigs for treatment and prophylaxis of proliferative enteropathies. In: Proceedings of the 21st IPVS Congress, Vancouver, Canada; 2010. p. 711.

71. Tzika ED, Tassis PD, Papatsiros VG, Koylialis D, Petridoy E, Alexopoulos C, *et al.* Evaluation of the in feed use of pumpkin for treatment and prophylaxis of *Escherichia coli* postweaning diarrhoea. In: Proceedings of the 21st IPVS Congress, Vancouver, Canada; 2010. p. 780.

72. Tzika ED, Tassis PD, Papatsiros VG, Koylialis D, Siochu A, Toyplikioti P, *et al*. Evaluation of the in feed use of Larch sawdust in sows for treatment and prophylaxis of postpartum dysgalactia syndrome. In: Proceedings of the 21st IPVS Congress, Vancouver, Canada; 2010. p. 1191.

73. Kantas D, Tzika ED, Papatsiros VG, Tassis PD, Kyriakis SC. Effect of a natural feed additive on performance and health of weaned piglets. In: Proceedings of 2nd Greek Veterinary Congress for Farm Animal Medicine, Food Safety and Hygiene, Consumer Protection. Thessaloniki, Greece; 2011. p. 152.

74. Penalver P, Huerta B, Borge C, Astorga R, Romero R, Perea A. Antimicrobial activity of five essential oils against origin strains of the Enterobacteriaceae family. APMIS 2005;113:1–6.

75. Fu Y, Zu Y, Chen L, Shi X, Wang Z, Sun S, *et al*. Antimicrobial activity of clove and rosemary oils alone and in combination. Phytotherapy Research 2007;21:989–94.

76. Barbosa LN, Rall VL, Fernandes AA, Ushimaru PI, da Sliva Probst I, Fernandes A Jr. Essential oils against foodborne pathogens and spoilage bacteria in minced meat. Foodborne Pathogen Diseases 2009;6:725–8.

77. Adams C. Nutricines: Food Components in Health and Nutrition. Nottingham University Press, UK; 1999.

78. Akagul A, Kivanc M. Inhibitory effects of selected Turkish spices and oregano compounds on some food-borne fungi. International Journal of Food Microbiology 1988;6:264–8.

79. Bruerton K. Antibiotic growth promoters – are there alternatives? In: Proceedings 2002 Poultry Information Exchange; 2002. pp. 171–6.

80. Barry TN, McNabb WC. The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. British Journal of Nutrition 1999;81:263–72.

81. Jouany J-P. Effect of rumen protozoa on nitrogen utilization by ruminants. Journal of Nutrition 1996;126:1335–46.

82. Mirzaei F, Hari Venkatesh KR. Efficacy of phyto medicines as supplement in feeding practices on ruminant's performance: a review. Global Journal of Research on Medicinal Plants and Indigenous Medicine 2012;1(9):391–403.

83. Waghorn GC, Ulyatt MJ, John A, Fisher MT. The effect of condensed tannins on the site of digestion of amino acids and other nutrients in sheep fed on *Lotus corniculatus* L. British Journal of Nutrition 1987;57:115–26.

84. Makkar HPS. Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. Small Ruminant Research 2003;49:241–56.

85. Rochfort S, Parker AJ, Dunshea FR. Plant bioactives for ruminant health and productivity. Phytochemistry 2008;69:299–322.

86. Bodas R, Lopez S, Fernandez M, Garcia-Gonzalez R, Wallace RJ, Gonzalez GS. Phytogenic additives to decrease *in vitro* ruminal methanogenesis. Options Méditerranéennes 2009;85:279–83.

87. Durmic Z, Blache D. Bioactive plants and plant products: effects on animal function, health and welfare. Animal Feed Science and Technology 2012;176:150–62.

88. Flachowsky G, Lebzien P. Effects of phytogenic substances on rumen fermentation and methane emissions: a proposal for a research process. Animal Feed Science and Technology 2012;176:70–7.

89. Woodward SI, Waghorn GC, Ulyatt MJ, Lassey KR. Early indications that feeding *Lotus* will reduce methane emissions from ruminants. Proceedings of the New Zealand Society of Animal Production 2001;61:23–6.

90. Waghorn GC, Tavendale MH, Woodfield DR. Methanogenesis from forages fed to sheep. Proceedings of the New Zealand Grasslands Association 2002;64:167–71.

91. Pinares-Patino CS, Ulyatt MJ, Waghorn GC, Lassey KR, Barry TN, Holmes CW, *et al*. Methane emission by alpaca and sheep fed on lucerne hay or grazed on pastures of perennial ryegrass/white clover or birdsfoot trefoil. Journal of Agricultural Science 2003;140:215–26.

92. Waghorn GC, Jones WT. Bloat in cattle. Potential of dock (*Rumex obtusifolius*) as an antibloating agent for cattle. New Zealand Journal of Agricultural Research 1989;32:227–35.

93. Min BR, Barry TN, Attwood GT, McNabb WC. The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: a review. Animal Feed Science and Technology 2003;106:3–19.

94. Min BR, Pinchak WE, Fulford JD, Puchala R. Wheat pasture bloat dynamics, *in vitro* ruminal gas production, and potential bloat mitigation with condensed tannins. Journal of Animal Science 2005;83:1322–31.

95. Butter NL, Dawson JM, Wakelin D, Buttery PJ. Effect of dietary tannin and protein concentration on nematode infection (*T. colubriformis*) in lambs. Journal of Agricultural Science, Cambridge 2000;134:89–99.

96. Athanasiadou S, Kyriazakis I. Plant secondary metabolites: antiparasitic effects and their role in ruminant production systems. Proceedings of the Nutrition Society 2004;63:631–39.

97. Sandoval-Castro CA, Torres-Acosta JFG, Hoste H, Salem AZM, Chan-Pérez JI. Using plant bioactive materials

to control gastrointestinal tract helminths in livestock. Animal Feed Science and Technology 2012;176:192–201.

98. Coop RL, Kyriazakis IK. Influence of host nutrition on the development and consequences of nematode parasitism in ruminants. Trends in Parasitology 2001;17:325–30.

99. Sen S, Makkar HPS, Becker K. Alfalfa saponins and their implication in animal nutrition. Journal of Agricultural and Food Chemistry 1998;46:131–40.

100. Milgate J, Roberts DCK. The nutritional and biological significance of saponins. Nutrition Research 1995;15:1223–49.

101. Cardozo PW, Calsamiglia S, Ferret A, Kamel C. Screening for the effects of natural plant extracts at different pH on *in vitro* rumen microbial fermentation of a high-concentrate diet for beef cattle. Journal of Animal Science 2005;83:2572–9.

102. Santoso B, Mwenya B, Sar C, Gamo Y, Kobayashi T, Morikawa R, *et al*. Effects of supplementing galactooligosaccharides, *Yucca schidigera* or nisin on rumen methanogenesis, nitrogen and energy metabolism in sheep. Livestock Production Science 2004;91:209–17.

103. Eryavuz A, Dehority BA. Effects of *Yucca schidigera* extract on the concentration of rumen microorganisms in sheep. Animal Feed Science and Technology 2004;117:215–22.

104. Mader TL, Brumm MC. Effect of feeding sarsaponin in cattle and swine diets. Journal of Animal Science 1987;65:9–15.

105. Makkar HPS, Becker K. Effect of pH, temperature, and time onin activation of tannins and possible implications in detannification studies. Journal of Agricultural and Food Chemistry 1996;44:1291–5.

106. Abreu A, Carulla JE, Lascano CE, Diaz TE, Kreuzer M, Hess HD. Effects of *Sapindus saponaria* fruits on ruminal fermentation and duodenal nitrogen flow of sheep fed a tropical grass diet with and without legume. Journal of Animal Science 2004;82:1392–400.

107. Hristov AN, Ivan M, Neill L, McAllister TA. A survey of potential bioactive agents for reducing protozoal activity *in vitro*. Animal Feed Science and Technology 2003;105:163–84.

108. Lila ZA, Mohammed N, Kanda S, Kamada T, Itabashi H. Effect of sarsaponin on ruminal fermentation with particular reference to methane production *in vitro*. Journal of Dairy Science 2003;86:3330–6.

109. Hu W, WU Y, Liu J, Guo Y, Ye J. Tea saponin affect *in vitro* fermentation and methanogenesis in faunated and defaunated rumen fluid. Journal of Zhejiang University Science 2005;6B:787–92.

110. Killeen GF, Madigan CA, Connolly CR, Walsh GA, Clark C, Hynes MJ, *et al*. Antimicrobial saponins of *Yucca schidigera* and the implications of their *in vitro* properties for their *in vivo* impact. Journal of Agricultural and Food Chemistry 1998;46:3178–86.

111. Wang Y, McAllister TA, Yanke LJ, Cheeke PR. Effect of steroidal saponin from *Yucca schidigera* extract on ruminal microbes. Journal of Applied Microbiology 2000;88:887–96.

112. Simons V, Morrissey JP, Latijnhouwers M, Csukai M, Cleaver A, Yarrow C, *et al*. Dual effects of plant steroidal alkaloids on *Saccharomyces cerevisiae*. Antimicrobial Agents and Chemotherapy 2006;50:2732–40.

113. McAllister TA, Annett C, Cockwill CL, Olson ME, Wang Y, Cheeke PR. Studies on the use of *Yucca schidigera* to control giardiosis. Veterinary Parasitology 2001;97:85–99.

114. Traore F, Faure R, Ollivier E, Gasquet M, Azas N, Debrauwer L, *et al*. Structure and antiprotozoal activity of triterpenoid saponins from *Glinus oppositifolius*. Planta Medica 2000;66:368–71.

115. Klita PT, Mathison GW, Fenton TW, Hardin RT. Effects of alfalfa root saponins on digestive function in sheep. Journal of Animal Science 1996;74:1144–56.

116. Newbold CJ, El Hassan SM, Wang J, Ortega M, Wallace RJ. Influence of foliage from African multipurpose trees on activity of rumen protozoa and bacteria. British Journal of Nutrition 1997;78:237–49.

117. Jouany J-P, Morgavi DP. Use of ‘natural’ products as alternatives to antibiotic feed additives in ruminant production Animal. Animal Consortium 2007;1(10):1443–66.

118. Griffin SG, Wyllie SG, Marham JL, Leach DN. The role of structure and molecular properties of terpenoids in determining their antimicrobial activity. Flavour and Fragrance Journal 1999;14:322–32.

119. Chao SC, Young DG, Oberg CG. Screening for inhibitory activity of essential oil on selected bacteria, fungi and viruses. Journal of Essential Oil Research 2000;12:639–49.

120. Burt S. Essential oils: their antibacterial properties and potential applications in foods – a review. International Journal of Food Microbiology 2004;94:223–53.

121. Davidson PM, Naidu AS. Phyto-phenols. In: Naidu AS, editor. Natural Food Antimicrobial Systems. CRC Press, Boca Raton, FL; 2000. p. 265–94.

122. Lee HG, Cheng SS, Chang ST. Antifungal property of the essential oils and their constituents from *Cinnamomum osmophloeum* leaf against tree pathogenic fungi. Journal of the Science of Food and Agriculture 2005;85:2047–53.

123. Tabanca N, Demirci B, Husnu Can Baser K, Aytac Z, Ekici M, Khan SI, *et al*. Chemical composition and antifungal activity of *Salvia macrochlamys* and *Salvia recognita* essential oils. Journal of Agricultural Food Chemistry 2006;54:6593–7.

124. Schnitzler P, Schon K, Reichling J. Antiviral activity of Australian tea tree oil and eucalyptus oil against herpes simplex virus in cell culture. Pharmazie 2001;56:343–7.

125. Farag RS, Shalaby AS, El-Baroty GA, Ibrahim NA, Ali MA, Hassan EM. Chemical and biological evaluation of the essential oils of different *Melaleuca* species. Phytotherapy Research 2004;18:30–5.

126. McIntosh FM, Williams P, Losa R, Wallace RJ, Beever DA, Newbold CJ. Effects of essential oils on ruminal microorganisms and their protein metabolism. Applied and Environmental Microbiology 2003;69:5011–4.

127. Newbold CJ, McIntosh FM, Williams P, Losa R, Wallace RJ. Effects of a specific blend of essential oil compounds on rumen fermentation. Animal Feed Science and Technology 2004;114:105–12.

128. Calsamiglia S, Busquet M, Cardozo PW, Castillejos L, Ferret A. Essential oils as modifiers of rumen microbial fermentation. Journal of Dairy Science 2007;90:2580–95.

129. Chaves AV, Dugan MER, Stanford K, Gibson LL, Bystrom JM, McAllister TA, *et al*. A dose-response of cinnamaldehyde supplementation on intake, ruminal fermentation, blood metabolites, growth performance,

and carcass characteristics of growing lambs. Livestock Science 2011;141:213–20.

130. Benchaar C, Greathead H. Essential oils and opportunities to mitigate enteric methane emissions from ruminants. Animal Feed Science and Technology 2011;166–167:338–55.

131. Benchaar C, Calsamiglia S, Chaves AV, Fraser GR, Colombatto D, McAllister TA, *et al*. A review of plant-derived essential oils in ruminant nutrition and production. Animal Feed Science and Technology 2008;145:209–28.

132. Benchaar C, Lettat A, Hassanat F, Yang WZ, Forster RJ, Petit HV, *et al.* Eugenol for dairy cows fed low or high concentrate diets: effects on digestion, ruminal fermentation characteristics, rumen microbial populations and milk fatty acid profile. Animal Feed Science and Technology 2012;178:139–50.

133. Yang WZ, Benchaar C, Ametaj BN, Beauchemin KA. Dose response to eugenol supplementation in growing beef cattle: ruminal fermentation and intestinal digestion. Animal Feed Science and Technology 2010;158:57–64.

134. Busquet M, Calsamiglia S, Ferret A, Carro MD, Kamel C. Effect of garlic oil and four of its compounds on rumen microbial fermentation. Journal of Dairy Science 2005;88:4393–404.

135. Duval SM, Newbold CJ, McEwan NR, Graham RC, Wallace RJ. Effect of a specific blend of essential oils on the colonization of substrates by rumen microorganisms. Reproduction Nutrition Development 2004;44(Suppl. 1):35.

136. Fuller R. Probiotics in man and animals. Journal of Applied Bacteriology 1989;66:365–8.

137. Sissons JW. Potential of probiotic organisms to prevent diarrhea and promote digestion in farm animals: a review. Journal of Food and Agriculture Science 1989;49:1–13.

138. Bomba A, Nemcova R, Gancarcikova S, Herich R, Guba P, Mudronova D. Improvement of the probiotic effect of microorganisms by their combination with maltodextrins, fructo-oligosacharides and polyunsaturated acids. British Journal of Nutrition 2002;88:95–9.

139. Guerra NP, Bernardez PF, Mendez J, Cachaldora P, Castro LP. Production of four potentially probiotic lactic acid bacteria and their evaluation as feed additives for weaned piglets. Animal Feed Science and Technology 1997;134:89–107.

140. Mantere Alhonen S. Propionibacteria used as probiotics – a review. Lait 1995;75:447–52.

141. Marinho MC, Lordelo MM, Cunha LF, Freire JPB. Microbial activity in the gut of piglets: i. Effect of prebiotic and probiotic supplementation. Livestock Science 2007;108:236–9.

142. Fooks LJ, Gibson GR. Probiotics as modulators of the gut flora. British Journal of Nutrition 2002;88(Suppl. 1):39–49.

143. Ouwehand AC, Salminen S, Isolauri E. Probiotics: an overview of beneficial effects. Antonie van Leeuwenhoek 2002;82:279–89.

144. Lodemann U, Hubener K, Jansen N, Martens H. Effects of *Enterococcus faecium* NCIMB 10415 as probiotic supplement on intestinal transport and barrier function of piglets. Archives of Animal Nutrition 2006;1:35–48.

145. Roselli M, Finamore A, Britti MS, Bosi P, Oswald I, Mengheri E. Alternatives to in-feed antibiotics in pigs: evaluation of probiotics, zinc or organic acids as protective agents for the intestinal mucosa. A comparison of *in vitro* and *in vivo* results. Animal Research 2005;54:203–18.

146. Shu Q, Qu F, Gill HS. Probiotic treatment using *Bifidobacterium lactis* HN019 reduces weaning diarrhea associated with rotavirus and *Escherichia coli* infection in a piglet model. Journal of Pediatric Gastroenterology Nutrition 2001;33:171–7.

147. Chiang BL, Sheih YH, Wang LH, Liao CK, Gill HS. Enhancing immunity by dietary consumption of a probiotic lactic acid bacterium (*Bifidobacterium lactis* HN019): optimization and definition of cellular immune responses. European Journal of Clinical Nutrition 2000;54:849–55.

148. Matsuzaki T, Chin J. Modulating immune responses with probiotic bacteria. Immunology and Cell Biology 2000;78:67–73.

149. Kyriakis SC, Tsiloyiannis VK, Vlemmas J, Sarris K, Tsinas AC, Alexopoulos C, *et al*. The effect of probiotic LSP 122 on the control of post-weaning diarrhoea syndrome of piglets. Research in Veterinary Science 1999;67:223–8.

150. Parks CW, Grimes JL, Ferket PR, Fairchild AS. The effect of mannan- oligosaccharides, bambermycin, and virginiamycin on performance of large white male mate turkeys. Poultry Science 2001;80:718–23.

151. Chen YJ, Kwon OS, Min BJ, Son KS, Cho JH, Hong JW, *et al*. The effects of dietary Biotite V supplementation as an alternative substance to antibiotics in growing pigs. Asian-Australasian Journal of Animal Sciences 2005;18:1642–5.

152. Alexopoulos C, Karagiannidis SK, Kritas SK, Boscos C, Georgoulakis IE, Kyriakis SC. Field evaluation of a bioregulator containing live *Bacillus cereus* spores on health status and performance of sows and their litters. Journal of Veterinary Medical Science 2001;48:137–45.

153. Scharek L, Altherr BJ, Tolke C, Schidt MFG. Influence of the probiotic *Bacillus cereus* var. *toyoi* on the intestinal immunity of piglets. Veterinary Immunology and Immunopathology 2007;120:136–47.

154. Scharek L, Guth J, Reiter K, Weyrauch KD, Tara D, Schwerk P, *et al*. Influence of a probiotic *Enterococcus faecium* strain on development of the immune system of sows and piglets. Veterinary Immunology and Immunopathology 2005;105:151–61.

155. Reiter K, Eggebrecht S, Drewes B, Riess M, Weyrauch KD. Effect of *Enterococcus faecium* and *Bacillus cereus* var. *toyoi* on the morphology of the intestinal mucous membrane in piglets. Biologia Bratislava 2006;61:1–7.

156. Ozawa K, Yokota H, Kimura M, Mitsuoka T. Effects of administration of *Bacillus subtilis* strain BN on intestinal flora of weanling piglets. Japanese Journal of Veterinary Science 1981;43:771–5.

157. Adami A, Cavazzoni V. Occurence of selected bacterial groups in the faeces of piglets fed with *Bacillus coagulans* as probiotic. Journal of Basic Microbiology 1999;39:3–9.

158. Link R, Kovac G, Pistl J. A note on probiotics as an alternative for antibiotics in pigs. Journal of Animal and Feed Sciences 2005;3:513–9.

159. Link R, Kovac G. The effect of probiotic BioPlus 2B on feed efficiency and metabolic parameters in swine. Biologia 2006;61:783–7.

160. Jin LZ, Zhao X. Intestinal receptors for adhesive fimbriae of enetrotoxigenic *Escherichia coli* (ETEC) K88 in swine – a review. Applied Microbiology and Biotechnology 2000;54:311–8.

161. Mathew AG, Chattin SE, Robbins CM, Golden DA. Effects of a direct-fed yeast culture on enteric microbial populations, fermentations acids, and performance of weanling pigs. Journal of Animal Science 1998;76:2138–45.

162. Roberfroid MB. Prebiotics and synbiotics: concepts and nutritional properties. British Journal of Nutrition 1998;80(Suppl. 2):197–202.

163. Duncan SH, Louis P, Flint HJ. Lactate-utilizing bacteria, isolated from human feces that produce butyrate as a major fermentation product. Applied Environmental Microbiology 2004;70:5810–7.

164. La Ragione RM, Narbad A, Gasson MJ, Woodward MJ. *In vivo* characterization of *Lactobacillus johnsonii* FI9785 for use as defined competitive exclusion agent against bacterial pathogens in poultry. Letters in Applied Microbiology 2004;38:197–205.

165. Hofacre CL, Froyman R, Gautrias B, George B, Goodwin MA, Brown J. Use of Aviguard and other intestinal bioproducts in experimental *Clostridium perfringens*-associated necrotizing enteritis in broiler chickens. Avian Diseases 1998;42:579–84.

166. Kalliomäki M, Salminen S, Isolauri E. Positive interactions with the microbiota: probiotics. Advances in Experimental Medical Biology 2008;635:57–66.

167. Ng SG, Hart AL, Kamm MA, Stagg AJ, Knight SC. Mechanism of action of probiotics: recent advances. Inflammatory Bowel Diseases 2009;15:300–10.

168. Madsen KL, Cornish A, Soper P, McKaigney C, Jijon H, Yachimec C, *et al*. Probiotic bacteria enhance murine and human intestinal epithelial barrier function. Gastroenterology 2001;121:580–91.

169. Sherman PM, Ossa JC, Johnson-Henry K. Unravelling mechanisms of action of probiotics. Nutrition Clinical Practice 2009;21:10–4.

170. Ellinger DK, Muller LD, Glandz PJ. Influence of feeding fermented colostrum and *Lactobacillus acidophilus* on faecal flora and selected blood parameters of young dairy calves. Journal of Dairy Science 1978;61(Suppl. 1):126.

171. Bruce BB, Gilliland SE, Bush LJ, Staley TE. Influence of feeding cattle cells of *Lactobacillus acidophilus* on faecal flora of young dairy calves. Annual Oklahoma Animal Science Research Report. 1979. p. 207

172. Bechman TJ, Chambers JV, Cunningham MD. Influence of *Lactobacillus acidophilus* on performance of young dairy calves. Journal of Dairy Science 1977;60(Suppl. 1):74.

173. Beeman K. The effect of Lactobacillus spp. on convalescing calves. Agri-practice 1985;6:8–10.

174. Wiryawan KG, Brooker JD. Probiotic control of lactate accumulation in acutely grain-fed sheep. Australian Journal of Agricultural Research 1995;46:1555–68.

175. Nocek JE, Kautz WP, Leedle JAZ, Allman JG. Ruminal supplementation of direct-fed microbials on diurnal pH variation and *in situ* digestion in dairy cattle. Journal of Dairy Science 2002;85:429–33.

176. Williams PEV, Tait CAG, Innes GM, Newbold CJ. Effects of the inclusion of yeast culture (*Saccharomyces cerevisiae* plus growth medium) in the diet of dairy cows on milk yield and forage degradation and fermentation patterns in the rumen of sheep and steers. Journal of Animal Science 1991;69:3016–26.

177. Durand-Chaucheyras F, Fonty G, Bertin G, Theveniot M, Gouet P. Fate of Levucell_R SCI-1077 yeast additive during digestive transit in lambs. Reproduction Nutrition Development 1998;38:275–80.

178. Lesmeister KE, Heinrichs AJ, Gabler MT. Effects of supplemental yeast (*Saccharomyces cerevisiae*) culture on rumen development, growth characteristics and blood parameters in neonatal dairy calves. Journal of Dairy Science 2004;87:1832–9.

179. Kawas JR, Garcia-Castillo R, Fimbres-Durazo H, Garza-Cazares F, Hernandez-Vidal JFG, Olivares-Saenz E, *et al*. Effects of sodium bicarbonate and yeast on nutrient intake, digestibility and ruminal fermentation of light-weight lambs fed finishing diets. Small Ruminant Research 2007; 67:149–56.

180. Fadel El-seed ANMA, Sekine J, Kamel HEM, Hishinuma M. Changes with time after feeding in ruminal pool sizes of cellular contents, crude protein, cellulose, hemicellulose and lignin. Indian Journal of Animal Sciences 2004;74:205–10.

181. Pal K, Paul SK, Bhunia T, Pakhira MC, Biswas P, Patra AK. Responses of addition of yeast (*Saccharomyces cerevisiae*) from rice distillers grains with solubles with or without trace minerals on the performance of Black Bengal kids. Small Ruminant Research 2010;94:45–52.

182. Jaquette RD, Dennis RJ, Coalson JA, Ware DR, Manfredi ET, Read PL. Effect of feeding viable *Lactobacillus acidophilus* (BT1386) on the performance of lactating dairy cows. Journal of Dairy Science 1988;71(Suppl. 1):219.

183. Ware DR, Read PL, Manfredi ET. Lactation performance of two large dairy herds fed *Lactobacillus acidophilus* strain BT 1386. Journal of Dairy Science 1988;71(Suppl. 1):219.

184. Jeong HY, Kim JS, Ahn BS, Cho WM, Kweon UG, Ha JK, *et al*. Effect of direct-fed microbials (DFM) on milk yield, rumen fermentation and microbial growth in lactating dairy cows. Korean Journal of Dairy Science 1998;20:247–52.

185. Savoini G, Mancin G, Rossi CS, Grittini A, Baldi A, Dell-Orto V. Administration of lactobacilli in transition [peripartum] cows: effects on blood level of glucose, beta-hydroxybutyrate and NEFA and on milk yield. Obiettivi e Documenti Veterinari 2000;21(1):65–70.

186. Oetzel GR, Emery KM, Kautz WP, Nocek JE. Direct-fed microbial supplementation on health and performance of pre- and postpartum dairy cattle: a field trial. Journal of Dairy Science 2007;90:2058–68.

187. Gibson GR, Beatty ER, Wang X, Cummings JH. Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. Gastroenterology 1995;108:975–82.

188. Iji PA, Tivey DR. Natural and synthetic oligosaccharides in broiler chicken diets. World Poultry Science Journal 1998;54:129–43.

189. Iji PA, Tivey DR. The use of oligosaccharides in broiler diets. In: Proceedings of the 12th European Symposium on Poultry Nutrition. WPSA, Dutch Branch, Veldhoven, The Netherlands; 1999. p. 193–201.

190. Flickinger EA, Van Loo J, Fahey GC. Nutritional response to the presence of inulin and oligofructose in the diets of domesticated animals: a review. Critical Reviews in Food Science and Nutrition 2003;43:19–60.

191. Patterson JA, Burkholder KM. Prebiotic feed additives: rationale and use in pigs. In: Proceedings of the 9th International Symposium on Digestive Physiology in pigs, Banff, Alberta, Canada; 2003. p. 319–31.

192. Grizard D, Barthomeuf C. Non-digestible oligosaccharides used as prebiotic agents: mode of production and beneficial effects on animal and human health. Reproduction Nutrition Development 1999;39:5–6.

193. Roberfroid nB, Bauer E, Mosenthin R. Pro- and prebiotics in pig nutrition – potential modulators of gut health? Journal of Animal and Feed Sciences 2001;10:47–56.

194. Tuohy KM, Rouzaud GCM, Bruck WM, Gibson GR. Modulation of the human gut microflora towards improved health using prebiotics-assessment of efficacy. Current Pharmaceutical Design 2005;11:75–90.

195. Vondruskova H, Slamova R, Trckova M, Zraly Z, Pavlik I. Alternatives to antibiotic growth promoters in prevention of diarrhoea in weaned piglets: a review. Veterinarni Medicina 2010;55(5):199–224.

196. Kumprecht I, Zobac P. Study of the effect of a combined preparation containing *Enterococcus faecium* M-74 and mannan-oligosacharides in diets for weanling piglets. Czech of Journal Animal Science 1998;43:477–81.

197. Shim SB, Verstegen WA, Kim IH, Kwon OS, Verdonk JMAJ. Effects of feeding antibiotic-free creep feed supplemented with oligofructose, probiotics or synbiotics to suckling piglets increases the preweaning weight gain and composition of intestinal microbiota. Archives of Animal Nutrition 2005;59:419–27.

198. Smiricky-Tjardes MR, Grieshop CM, Flickinger EA, Bauer LL, Fahey GC. Dietary galactooligosaccharides affect ileal and total-tract nutrient digestibility, ileal and fecal bacterial concentrations, and ileal fermentative characterisctics of growing pigs. Journal of Animal Science 2003;81:2535–45.

199. Crittenden RG, Playne MJ. Production, properties, and applications of food-grade oligosaccharides. Trends in Food Science and Technology 1996;7:353–61.

200. McCann MEE, Newell E, Preston C, Forbes K. The use of mannan-oligosaccharides and /or tannin in broiler diets. International Journal of Poultry Science 2006;5(9):873–9.

201. Oyofo BA, Droleskey RE, Norman JO, Mollenhauer HH, Ziprin RL, Corrier DE, *et al*. Inhibition by mannose of *in vitro* colonization of chicken small intestine by *Salmonella typhimurium*. Poultry Science 1989a;68:1351–6.

202. Oyofo BA, DeLoach JR, Corrier DE, Norman JO, Ziprin RL, Mollenhauer HH. Prevention of *Salmonella typhimurium* colonization of broilers with d-Mannose. Poultry Science 1989b;68:1357–60.

203. Fukata T, Sasai K, Miyamoto T, Baba E. Inhibitory effects of competitive exclusion and fructooligosaccharide, singly, and in combination, on Salmonella colonization of chicks. Journal of Food Protection 1999;62:229–33.

204. Xu ZR, Hu CH, Xia MS, Zhan XA, Wang MQ. Effects of dietary frutooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. Poultry Science 2003;82:1030–6.

205. Waldroup AL, Skinner JT, Hierholzer RE, Waldroup PW. An evaluation of fructo-oligosaccharide in diets for broiler chickens and effects on Salmonellae contamination of carcasses. Poultry Science 1993;72:643–50.

206. Verdonk JMAJ, Shim SB, van Leeuwen P, Verstegen WA. Application of inulin-type fructans in animal feed and pet food. British Journal of Nutrition 2005;93(Suppl. 1):125–38.

207. Ramirez-Farias C, Slezk K, Fuller Z, Duncan A, Holtrop G, Louis P. Effect on inulin on the human gut microbiota: stimulation of *Bifidobacterium adolescentis* and *Faecalibacterium prausnitzii*. British Journal of Nutrition 2009;101:541–50.

208. Ofek I, Mirelman D, Sharon N. Adherence of *Escherichia coli* to human mucosal cells mediated by mannose receptors. Nature (London) 1977;265:623–5.

209. Spring P, Wenk C, Dawson KA, Newman KE. The effects of dietary mannan-oligosaccharides on caecal parameters and the concentrations of enteric bacteria in the caeca of *Salmonella*-challenged broiler chicks. Poultry Science 2000;79:205–11.

210. Fernandez F, Hinton M, Van Gils B. Dietary mannan-oligosacccharides and their effect on chicken caecal microflora in relation to *Salmonella enteritidis* colonisation. Avian Pathology 2002;31:49–58.

211. Schepetkin IA, Quinn MT. Botanical polysaccharides: macrophage immunomodulation and therapeutic potential. International Immunopharmacology 2006;6:317–33.

212. Novak M, Vetvicka V. Beta-glucans, history, and the present: immunomodulatory aspects and mechanisms of action. Journal of Immunotoxicology 2008;5:47–57.

213. Nestorov N. Possible applications of natural zeolites in animal husbandry. In: Pond WG, Mumpton FA, editors. Zeo-Agriculture: Use of Natural Zeolites in Agriculture and Aquaculture. Westview Press Inc., Boulder, CO; 1984. p. 167–74.

214. Yannakopoulos A, Tserveni-Gousi A, Kassoli-Fournaraki A, Tsirabides A, Michailidis K, Filippidis A, *et al.* Effects of dietary clinoptilolite rich-tuff on the performance of growing-finishing pigs. In: Colella C, Mumpton FA, editors. Natural Zeolites for the Third Millennium. De Frede Editore, Napoli, Italy; 2000. p. 471–81.

215. Shurson GC, Ku PK, Miller ER, Yokohama MT. Effects of zeolite A or clinoptilolite in diets of growing swine. Journal of Animal Science 1984;59:1536–45.

216. Papaioannou DS, Kyriakis SC, Papasteriadis A, Roumbies N, Yannakopoulos A, Alexopoulos C. Effect of in-feed inclusion of a natural zeolite (clinoptilolite) on certain vitamin, macro and trace element concentrations in the blood, liver and kidney tissues of sows. Research in Veterinary Science 2002;72:1–8.

217. Yannakopoulos A, Tserveni-Gousi A, Fortomaris P, Arsenos G, Filippidis A, Kassoli-Fournaraki A. Effects of dietary inclusion of natural Greek zeolite on the reproductive characteristics of sows. In: Misaelides P, editor. Zeolite ‘02, Proceedings of 6th Int. Conf. Occurrence, Properties and Utilization of Natural Zeolites, Thessaloniki, Greece; 2002. p. 393.

218. Olver MD. Effect of feeding clinoptilolite (zeolite) on the performance of three strains of laying hens. British Poultry Science 1997;38:220–2.

219. Yannakopoulos AL, Tserveni-Gousi AS, Katsaounis NK, Kassoli-Fournaraki A, Filippidis A, Tsolakidou A. The influence of Greek clinoptilolite-bearing rocks on the performance of laying hens, in the early stage of laying.

In: International Symposium and Exhibition of Natural Zeolites, Sofia, Bulgaria; 1995. p. 120.

220. Tserveni-Gousi AS, Yannakopoulos AL, Katsaounis NK, Filippidis A, Kassoli-Fournaraki A. Some interior egg characteristics as influenced by addition of Greek clinoptilolite rock material in the hen diet. Archive Geflügelk 1997;61:191–296.

221. Mirabdolbaghi J, Lotfollahian DK. The effect of natural and processed Iranian zeolite on broiler chicken performance. In: Misaelides P, editor. Zeolite ’02, Proceedings of 6th Int. Conf. Occurrence, Properties and Utilization of Natural Zeolites, Thessaloniki, Greece; 2002. p. 234.

222. Lotfollahian H, Mehdizadeh S, Mirabdolbaghi J. Effects of two kinds of Iranian natural zeolite on broiler performance. In: XVIth European Symposium on the Quality of Poultry Meat, Xth European Symposium on the Quality of Eggs and Egg Product; 2003. p. 17–9.

223. Christaki E, Florou-Paneri P, Tserveni-Gousi A, Yannakopoulos A, Fortomaris P. Effects of dietary inclusion of natural zeolite on broiler performance and carcass characteristics. In: Galarneau A, Di Rienzo F, Fajuala F, Vedrine J, editors. Studies in Surface Science and Catalysis 135. Zeolites and Mesoporous Materials at the Dawn of the 21st Century. Elsevier Science BV; 2001. p. 1–7.

224. Christaki E, Florou-Paneri P, Fortomaris P, Tserveni-Gousi A, Yannakopoulos A. Effects of dietary inclusion of natural zeolite and flaxseed on body fat deposition in broiler chickens. In: Misaelides P, editor. Zeolite ’02, Proceedings of 6th Int. Conf. Occurrence, Properties and Utilization of Natural Zeolites, Thessaloniki, Greece; 2002. p. 61.

225. Dedousi A, Georgopoulou I, Christaki E, Yannakopoulos A, Tserveni-Goussi A. The effect of natural zeolite (clinoptilolite) on total bacteria contamination of ostrich eggshells. Archive fur Geflugelkunde 2008;73(4):157–63.

226. Filippidis A, Godelitsas A, Charistos D, Misaelides P, Kassoli-Fournaraki A. The chemical behaviour of natural zeolites in aquaeous environments: interactions between low-silica zeolites and 1 M NaCl solutions of different initial pH-values. Applied Clay Science 1996;11:199–209.

227. Karatzia MA. Effect of dietary inclusion of clinoptilolite on antibody production by dairy cows vaccinated against *Escherichia coli*. Livestock Science 2010;128:149–53.

228. Karatzia MA, Pourliotis K, Katsoulos PD, Karatzias H. Effects of in-feed inclusion of clinoptilolite on blood serum concentrations of aluminium and inorganic phosphorus and on ruminal pH and volatile fatty acid concentrations in dairy cows. Biological Trace Element Research 2011;142:159–66.

229. Dschaak CM, Eun J-S, Young AJ, Stott RD, Peterson S. Effects of supplementation of natural zeolite on intake, digestion, ruminal fermentation, and lactational performance of dairy cows. Professional Animal Scientist 2010;26:647–54.

230. Fratric N, Stojic V, Jankovic D, Samanc H, Gvozdic D. The effect of a clinoptilolite based mineral adsorber on concentrations of immunoglobulin G in the serum of newborn calves fed different amounts of colostrum. Acta veterinaria (Beograd) 2005;55:11–21.

231. Fratric N, Stojic V, Rajcic V, Radojicic B. The effect of mineral adsorbent in calf diet colostrum on the levels of serum immunoglobulin g, protein and glucose. Acta veterinaria (Beograd) 2007;57:169–80.

232. Gvozdic D, Stojic V, Samanc H, Fratric N, Dacovic A. Apparent efficiency of immunoglobulin absorption in newborn calves orally treated with zeolite. Acta veterinaria (Beograd) 2008;58:345–55.

233. Pourliotis K, Karatzia MA, Florou-Paneri P, Katsoulos PD, Karatzias H. Effects of dietary inclusion of clinoptilolite in colostrum and milk of dairy calves on absorption of antibodies against *Escherichia coli* and the incidence of diarrhea. Animal Feed Science and Technology 2012;172:136–40.

234. Alcala-Canto Y, Gutierrez-Olvera L, Gutierrez-Olvera C, Sumano-Lopez H. Effects of clinoptilolite on *Eimeria* spp. Infection in sheep. Small Ruminant Research 2011;100(2):184–8.

235. Deligiannis K, Lainas T, Arsenos G, Papadopoulos E, Fortomaris P, Kufidis D, *et al*. The effect of feeding clinoptilolite on food intake and performance of growing lambs infected or not with gastrointestinal nematodes. Livestock Production Science 2005;96:195–203.

236. Katsoulos PD, Panousis N, Roubies N, Christaki E, Arsenos G, Karatzias H. Effects of long-term feeding of a diet supplemented with clinoptilolite to dairy cows on the incidence of ketosis, milk yield, and liver function. Veterinary Record 2006;159:415–8.