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MINI-REVIEW

Health benefits observed after probiotic *Lactobacillus fermentum* CCM 7421 application in dogs

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Abstract The importance of the intestinal microbiota has attracted much interest in recent years particularly with respect to ways in which the microbiota can be manipulated in order to improve health. Improving gut health through the use of probiotic microorganisms has become an area of research activity in both human and animal nutrition. However, the amount of research using companion animals is insufficient. The present review evaluates and compares the effects achieved after application of canine-derived probiotic strain *Lactobacillus fermentum* CCM 7421 to healthy dogs as well as to dogs suffering from gastrointestinal disorders. The experiments involved varying duration of application (4 days–14 days), dosage (10^7–10^9 CFU), form of application (fresh culture or lyophilized from) or combination with natural substances. Results from nine independent studies show the ability of probiotic strains to establish themselves in the canine gastrointestinal tract, alter the composition of intestinal microbiota and metabolites (organic acids), and modulate the physiology (serum biochemical parameters) and immunity parameters in dogs. Almost all observed effects were irrespective of dose or duration of probiotic administration.

Keywords Probiotics · Effect · Canine study · Gastrointestinal tract · Microbial-host interactions

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Introduction

The dog was the first domesticated animal species and together with cats belongs among the most popular companion animals worldwide. This popularity is still increasing, as shown by the latest available statistics for Europe (increase of over 5 million dogs from 2012 to 2014; Statista 2016). An increase in multiple dog-owning households has also contributed to the overall growth in dog ownership. Although the total population in Europe reaches approximately 81 million, the number of dogs per person varies from country to country. Lithuania, Romania, Poland and the Czech Republic are countries with the highest number of dogs per person: one dog per four–five persons (FEDIAF 2014). In Slovakia, 898,000 dogs was estimated (one dog per six persons), which means that every other household keeps a dog. The reason for the large expansion in dog breeding lies in their valuable properties such as intelligence, social behaviour and better developed sense organs (smell, hearing) than in humans, and they therefore perform a variety of roles in human society and are often trained as working dogs. Moreover, domestic dogs have been selectively bred for millennia for various behaviours, sensory capabilities and physical attributes, resulting in a broad spectrum of different breeds. Nowadays, the international canine organisation, the Fédération Cynologique Internationale recognizes 344 breeds of dogs (FCI 2016). An important aspect of dog ownership lies also in the health benefits such as stress reduction and relaxation promotion, impact on social, emotional and cognitive development in children or promotion of an active lifestyle (Harvard Medical School 2015). Other health benefits include protection against allergies, which is in accordance with the hygiene hypothesis (Fall et al. 2015) or reduction of risk of cardiovascular diseases (Levine et al. 2013). The priority of every responsible dog owner is to enable their dog to lead a healthy lifestyle with plenty of physical activity, grooming and high-quality food. Failure to comply with these conditions is often one of the main causes of canine diseases. While much progress has been made in the molecular characterisation of inherited diseases in dogs (the number of available DNA tests is growing exponentially) and thus their prevalence has started being controlled (Slutsky et al. 2013), the incidence of special acquired diseases or disorders is still increasing. The most prevalent disorder groups recorded in dogs in recent years are dermatological, enteropathic and musculoskeletal problems (O Neill et al. 2014; VPI 2015). The head and neck was the body area most frequently affected, and inflammation was the most prevalent pathophysiological process in the disorders recorded. Interestingly, otitis externa and allergic dermatitis are very frequently diagnosed and belong among the leading medical conditions. The crucial role in the majority of these diseases is played by disruption of intestinal microbiota equilibrium (dysbiosis) and the immune system (Craig and Cert 2016; Suchodolski 2016).

Dysbiosis can be defined as any change in the composition of resident commensal communities relative to the community found in healthy individuals (Petersen and Round 2014). There are three types of dysbiosis discussed, such as (i) loss of beneficial microorganisms, (ii) expansion of pathobionts or potentially harmful microorganisms and (iii) loss of overall microbial diversity. Especially bacterial groups within the phyla Firmicutes and Bacteriodetes, which are reduced during intestinal inflammation (Honneffer et al. 2014), seem to be important in maintenance of gastrointestinal or overall health also in dogs. Although limited information is available about the functional consequences associated with dysbiosis, impairment of the intestinal mucosal barrier often follows microbiome changes (Craig and Cert 2016). Enhanced transfer of intact and degraded proteins through this barrier increases the antigenic load and could explain the frequent occurence of allergic diseases in dogs. The reasons for the development of dysbiosis are more complex and include dietary factors (food changes, high level of fermentable carbohydrates) as well as medical interventions (antibiotics, non-steroidal anti-inflammatory drugs, gastric acid supressants), infections, individual genetics, hormonal changes, environmental factors (contaminated water and food, chemical additivesartificial colours and flavours, emulsifiers, antioxidants), and psychological and physical stress. The link between gastrointestinal function and the central nervous system (CNS) was first postulated over 80 years ago, but recently many aspects of this gut-brain axis have been validated (Bercik et al. 2012; Carabotti et al. 2015). Signalling molecules released into the gut lumen from cells in the lamina propria which are controlled by the CNS can result in changes in gastrointestinal motility and secretion as well as intestinal permeability, thus altering the gastrointestinal environment in which the bacteria reside. Emotional stress in dogs (mostly due to change of housing, transportation, competitions, being left at home alone for longer periods) should therefore not be underrated, since it can contribute to dysbiosis.

The efforts of the research community are aimed at discovering microbial or non-microbial natural alternatives for assurance of intestinal microflora stability and for increased counts of beneficial microorganisms. The use of probiotics and prebiotics, or their combination in synbiotics, and maybe also combinations of probiotics with other synergistically acting components of natural origin (herbal extracts, organic acids, polyunsaturated fatty acids) called potentiated probiotics are among the most promising methods (Bomba et al. 2006). However, the majority of knowledge about probiotic microorganisms and prebiotic substances relates to the area of human medicine (mostly strains of human commensal or food origin; Gallego and Salminen 2016; Pandey et al. 2015), while a lot of studies have also indicated their positive effects on weight gain, food efficiency, productivity, frequency of diarrhoea, the immune system and quantitative composition of intestinal microflora after application to farm animals (Chaucheyras-Durand and Durand 2010). Concerning companion animals, the clinical effects of probiotics and prebiotics have only started being explored (Grześkowiak et al. 2015). Results of several canine studies indicate that specific probiotic bacteria can modulate immune functions (Benyacoub et al. 2003), show anti-inflammatory effects (Kainulainen et al. 2015; Rossi et al. 2014,), normalize dysbiosis or reduce harmful bacteria (*Clostridium* spp., *Salmonella* sp.; Baillon et al. 2004; Maia et al. 2001; Rossi et al. 2014; Sauter et al. 2006; Vahjen and Männer 2003) and improve haematological or serum biochemical parameters (Baillon et al. 2004, Marciňáková et al. 2006). However, most of these studies and the probiotic products commercially available for dogs on the market use probiotic microorganisms of human origin (*Lactobacillus rhamnosus* GG, *L. acidophilus* DSM13241, *Enterococcus faecium* SF68, *E. faecium* M-74 etc.), which often do not show the full extent of expected effects in dogs, do not survive gastrointestinal transit in adequate numbers to enable any possible positive effect, or do not colonize the canine intestine for a longer time after cessation of their administration (Weese and Anderson 2002). Probiotic strains have been shown to have strain-specific and host species-specific actions (Shida et al. 2011), but according to the FAO/WHO guideline for probiotic evaluation (2006), the specificity of the action of a probiotic should be assessed as important, rather than its source.

The aim of this review is to summarize all achieved physiological and clinical effects of the probiotic strain *Lactobacillus fermentum* CCM 7421 (AD1 previous working labelling, Strompfová et al., 2006) isolated in 2001 from the faeces of healthy dog in the Laboratory of Animal Microbiology of Institute of Animal Physiology (Slovak Academy of Sciences) and tested to date in over 100 dogs and poultry species (turkeys, Japanese quails). The experiments involved varying (i) duration of application (4–14 days), (ii) dosage (10^7–10^9 CFU), (iii) form of application (fresh culture or lyophilized from) or (iv) combination with natural substances (inulin, plant extract *Eleutherococcus senticosus*, chlorophyll, alginite). The CCM 7421 strain was approved for patenting by the Industrial Property Office of the Slovak Republic in 2012, and it is currently available as ProBioDog probiotic product made by the International Probiotic Company located in Košice (Slovakia), also covering the markets in neighbouring states.

Effects of *Lactobacillus fermentum* CCM 7421 observed in experiments

Effect on faecal microbiota

In all canine experiments, application of *L. fermentum* CCM 7421 was associated with increased lactic acid bacteria counts (determined by culture techniques) during the treatment period by 1.4 log cycle on average (achieving statistical significance in almost all studies, Table 1). The use of different forms of probiotic strain (fresh vs. lyophilized) as well as different doses (10^7–10^9 CFU) had almost no impact on the increase rate (Strompfová et al. 2012a). Some of these experiments testing the forward-time effect after cessation of CCM 7421 strain application showed temporary decrease (to baseline counts) in the first week followed by an increase returning to levels recorded during application (2–5 weeks of post-treatment period, Strompfová et al. 2012a). The probiotic strain CCM 7421 was able to colonize the digestive tract of dogs, whereby faecal levels of 10^5–10^6 CFU/g during application and 10^2–10^4 CFU/g in the post-treatment period were most commonly detected. Although the counts of this strain were lower in the period following cessation of its administration, it was still able to persist for several weeks in the intestine of most dogs (five post-treatment weeks tested). An important point could be the fact that maximum counts of probiotic strain were already observed in the first week of application (Strompfová et al. 2012a, 2015) while longer application did not produce any further increase in strain counts in faecal samples. This could lead to consideration of whether short-term/one-off application with regular character is not more appropriate than long-term daily application of this strain especially from the preventative point of view (based also on other parameter results in the post-treatment period). One frequently-observed significant result was reduction of the clostridial population at the end of the treatment period and continuing 1–2 weeks into the post-treatment phase (by 0.9 log on average, Strompfová et al. 2012a, 2013). Another no less important result concerns the Gram-negative bacteria population tested in our experiments. Significant reduction of *Escherichia coli* counts was noted in a group of dogs with initially higher counts of these bacteria (reduced by 1.3 log, Strompfová et al. 2012a), although they were also reduced in other experiments to some extent (by 0.7–1.1 log, Strompfová et al. 2007, 2012a, 2012b). The CCM 7421 strain seems to be very useful especially in dogs suffering from diarrhoeal conditions involving coliform bacteria, because their counts in dogs with initial levels of $10^{9–11}$ CFU/g in faeces were reduced by over 3 log cycles after 7-day probiotic application (Strompfová et al., 2007). Treatment effects are therefore often more visible in individual dogs, with the necessity to modify their intestinal microbiota, rather than in the bacterial count average of the dog group. Other Gram-negative bacteria tested in several canine studies such as *Pseudomonas* spp. or *Aeromonas* spp. were slightly reduced (by 0.5–1.1 log, Strompfová et al. 2012a, 2013).

In general, there is a lack of probiotic studies testing the kinetics of intestinal colonization and/or the influence of probiotic strain on intestinal bacterial populations detected in more samplings during treatment and post-treatment periods, as well as trials regarding the relationship with dose or duration of treatment. The concentration of probiotics needed to obtain a clinical effect is often quoted as $\geq 10^6$ CFU/ml in the small intestine and $\geq 10^8$ CFU/g in the colon (Minelli and Benini 2008). Although not all, many studies especially in acute infectious diarrhoea showed dose-dependent effects and it seems that usual effective dosage in humans is 10^7–10^9 CFU/mg per day. Paradoxically, we observed that a lower dose or shorter treatment with probiotic had the same or more significant microbial effects than higher doses and longer treatment (Strompfová et al., 2012a). Similarly Zhang et al. (2016) reported after their meta-analysis of probiotic treatment of irritable bowel syndrome in humans that lower doses and shorter treatment duration were more effective with respect to overall symptom response. On the other hand, there are reports of clinical conditions such as antibiotic-associated diarrhoea where only higher doses (>10^{10} CFU) have been effective (Ouwehand 2016). In our clinical study, a dose of 10^9 CFU and 7-day treatment was sufficient to improve diarrhoea symptoms, microbiota composition or modify some serum biochemical parameters in dogs (Strompfová et al. 2007). Increase in the lactic acid bacteria population after application of probiotic lactobacilli is a commonly observed fact also in dogs (Baillon et al. 2004; Biagi et al. 2007; Sauter et al., 2006) and could be attributed to the ability of supplemented lactobacilli to colonize the intestine. However, there are canine studies where despite supplementation and survival of probiotic lactobacilli in the intestine, no increase was noted (Manninen et al. 2006). An effective probiotic has to colonize the gut mucosa at least temporarily (Salminen et al. 1998), which the strain *L. fermentum* CCM 7421 manage (persistence over several weeks in most dogs), in contrast to other strains with rapid clearance from the colon in a few days irrespective of their origin (Baillon et al. 2004; Manninen et al., 2006; Weese and Anderson, 2002). The

Table 1 Quantitative microbial changes in faeces after application of *L. fermentum* CCM 7421 alone or in combination with additive in dogs (evaluated by repeated measures ANOVA or *t* test in two sampling studies)

Dosage	Used form	Length of application	Tested microorganism					Reference
			Coliforms	LAB	Enterococci	Clostridia	CCM 7421	
10^8	Fresh	4 days[a]	Decreased	Increased	Increased	Not tested	$>10^5$	Strompfová et al. 2005
10^9	Fresh	7 days	Unchanged	Increased	Increased	Not tested	$>10^7$	Strompfová et al. 2006
10^9	Fresh	7 days	Unchanged	Increased	Increased	Not tested	$>10^7$	Strompfová et al. 2007
10^7	Lyophilized	7 days	Decreased	Increased	Unchanged	Not tested	Not tested	Strompfová et al. 2012a
10^8	Fresh	14 days	Unchanged	Trend for increase	Unchanged	Unchanged	$>10^5$	Strompfová et al. 2015
10^8	Fresh	14 days	Unchanged	Increased	Unchanged	Decreased	10^4–10^5	Strompfová et al. 2012b
10^8	Fresh	14 days	Unchanged	Increased	Unchanged	Decreased	10^4–10^5	Strompfová et al. 2013
10^8	Fresh	14 days	Decreased	Increased	Unchanged	Decreased	$>10^5$	Kubašová et al. 2016
10^8	Lyophilized	14 days	Unchanged	Increased	Unchanged	Decreased	$>10^5$	Strompfová et al. 2012a
L. fermentum CCM 7421 + additive								
10^8	Fresh + inulin (1% of diet)	14 days	Decreased	Increased	Unchanged	Unchanged	$>10^5$	Strompfová et al. 2013
10^8	Fresh + *E. senticosus* (8 mg/kg BW)	14 days	Unchanged	Increased	Unchanged	Unchanged	10^4–10^5	Strompfová et al. 2012b
10^8	Fresh+chlorophyllin (60 mg/dog)	14 days	Trend for decrease	Unchanged	Unchanged	Decreased	$>10^4$	Strompfová et al. 2015
10^8	Fresh + alginite (1% of diet)	14 days	Decreased	Trend for increase	Trend for increase	Decreased	10^3–10^5	Kubašová et al. 2016

[a] Tested in Japanese quails

suppression of clostridia counts after lactic acid bacteria consumption has been detected in several previous canine studies (Baillon et al. 2004; O Mahony et al. 2009; Vahjen and Männer 2003). However, clostridia are also commonly found in healthy dogs, though their numbers are higher in dogs with diarrhoea. Among them, *Clostridium perfringens* and *C. difficile* are enteropathogens of great importance (Marks et al. 2011). Various pathogen (strain) and host factors (age, immune status, antimicrobial exposure and comorbidities) probably play a key role in determining whether colonization progresses to disease. The important virulence factor of these clostridial species is production of toxins, while nontoxigenic strains are considered clinically irrelevant. Clinical signs range from subclinical carriage to potentially fatal acute haemorrhagic diarrhoeal syndrome (Cave et al. 2002). Although there are few studies specifically investigating the mechanisms of *Clostridium* spp. reduction during probiotic consumption, animal and cell culture studies have demostrated that some probiotic strains can inhibit their growth, block the attachment sites for clostridia, regulate immune responses or hydrolyse pathogenic toxins (A or B of *C. difficile*; Chen et al. 2013). Even the in vitro study by Grześkowiak et al. (2014) using canine lactobacilli showed higher pathogen exclusion with probiotics deactivated by heat compared to their viable forms. *E. coli* is similarly as clostridia part of the normal intestinal microbiota, but can be associated with gastroenteritis in the presence of bacterial virulence factors and impaired local or systemic immunity. Several distinct pathotypes of diarrheogenic *E. coli* are now recognized (enteropathogenic-EPEC, enterotoxigenic-ETEC, enterohemorrhagic-EHEC, necrotoxigenic-NTEC, enteroinvasive-EIEC, enteroaggregative-EAEC and adherent-invasive-AIEC strains). Many strains have been isolated from dogs with and without diarrhoea, and the role of many of these strains in disease causation in dogs is poorly defined. Granulomatous colitis was the most commonly observed manifestation in dogs (Marks et al. 2011). Counts of *Enterobacteriaceae* decreased in many probiotic studies using healthy dogs or dogs with gastrointestinal diseases (Sauter et al. 2006; Strompfová et al. 2014), and mechanisms such as (i) enhancement of the epithelial barrier (e.g. increased tight junction protein expression, or increased mucin expression), (ii) inhibition of adhesion (e.g. qualitative alterations in intestinal mucins preventing pathogen binding, or release of defensins from epithelial cells), (iii) competitive exclusion of pathogens (creation of a hostile microecology, elimination of available bacterial receptor sites, competitive depletion of essential nutrients), (iv) production of anti-microorganism substances (organic acids, bacteriocins) and (v) modulation of the immune system may be involved (Bermudez-Brito et al. 2012). Above all, organic acids, in particular acetic acid and lactic acid, have a strong inhibitory effect against Gram-negative bacteria, and they have been proposed as the main antimicrobial compounds responsible for the inhibitory activity of probiotics against these bacteria. Non-dissociated organic acids are lipophilic and can passively diffuse across bacterial cell membranes, and once internalized into the neutral pH of the cell cytoplasm they dissociate into anions and protons, both of which exert an inhibitory effect on bacteria. Releasing proton ions causes the internal pH to decrease, leading to disruption of proton motive force, and inhibiting substrate transport mechanisms and enzymatic reactions (Ricke 2003). Normalization of the intestinal microbiota using probiotic microorganisms seems to be an effective way especially in dogs with gastrointestinal pathogens, although stabilization in clinically healthy dogs is no less important from the preventative point of view.

Effect on production of organic acids and faecal pH

The primary fermentative end-products produced from dietary fibre are short-chain fatty acids (SCFA), predominantly acetate, propionate and butyrate. The SCFAs produced are rapidly absorbed from the intestinal lumen, with 95–99% being absorbed before reaching the distal colon (von Engelhardt, 1995). The great inter-individual differences in concentrations of organic acids most probably reflect different food/substrate and microbial population compositions in specific individuals. For this reason, measurement of organic acid concentrations in faeces has no great information value, and it is often omitted in canine studies. Application of *L. fermentum* CCM 7421 to dogs led to increased levels (significant or tendency) of many tested organic acids during treatment, even persisting for 1–2 weeks after probiotic application ceased (Strompfová et al. 2012a; Strompfová et al. 2015). Among them, the concentrations of lactic, butyric and succinic acids were most often increased in faecal samples from dogs (Fig. 1). Similarly, significant increase in lactic acid in small intestine contents was detected in Japanese quails after 4-day application of this probiotic (Strompfová et al. 2005). Notably, a few days' application of fresh probiotic culture was enough for instant increase in acid concentrations. Another interesting fact is the persistence of this effect, which could be related to slower absorbance of lactic or succinic acid (absorbed by a mechanism that is different from that of SCFA) leading to accumulation of these acids (Umesaki et al. 1978). The study by Biagi et al. (2007) using canine-derived *L. animalis* LA4 strain showed no changes in SCFA faecal concentration after 10 days of strain application to dogs, whereas significant increase in lactic acid was measured in vitro in dog faecal cultures (at 4 h). The main function of SCFA is to supply colonocytes with energy (approximately 7% of the basal energy requirement in dogs, Herschel et al. 1981) through their oxidizing in the order of butyric > propionic > acetic acid and to stimulate water and electrolyte resorption (Clausen and Mortensen 1994). Increasing mesenteric blood flow, mineral absorption and antimicrobial action are further important effects. Individual SCFA may have specific roles such as stimulation of apoptosis in tumour cell lines, regulation of inflammatory responses, influence on epithelial cell proliferation and differentation, enhancement of endogenous enzyme activity, modulation of the enteric neuroendocrine system, impact on the gastrointestinal motility, and on gluconeogenesis, lipogenesis and cholesterogenesis (Den Besten et al. 2013). The efficacy of an acid in inhibiting microbes depends on its pKa value, which is the pH at which 50% of the acid is dissociated. Organic acids with higher pKa values are more effective inhibitors, and their antimicrobial efficacy generally improves with increasing chain length and degree of unsaturation (Foegeding and Busta 1991). In addition, each acid has its own spectrum of antimicrobial activity: whereas lactic acid is more effective in reducing coliforms, clostridia, listeria and *Salmonella* sp., other acids such as formic or propionic have broader antimicrobial activities including regulation of fungi and yeasts (Knarreborg et al. 2002; Raftari et al. 2009). Accordingly, the intestinal pH value is one of the inhibitory factors which can limit the growth of bacteria. Significant decrease or trend towards decrease in faecal pH in dogs was measured at the end of *L. fermentum* CCM 7421 application continuing for 2 weeks after cessation of its administration (independently of the form of application, Strompfová et al., 2012a, 2015). Although the decrease in faecal pH corresponds with decrease in colifom bacteria in most of our canine studies, it seems that further mechanisms of reduction of these bacteria are also involved, because application of *L. fermentum* CCM 7421 to Japanese quails caused no change in intestinal pH despite significant reduction of *E. coli* (Strompfová et al., 2005). It is necessary to take into account the amount of organic acid produced during needful long-term applications of probiotic strains, which produce many heath benefits on the one hand, but overproduction of which (especially with diets rich in saccharides) on the other hand, could shift the acid-base balance. Although a variety of microbes in the gut, including probiotics, are known to produce lactic acid (L- and D-isomers), it is the elevated production

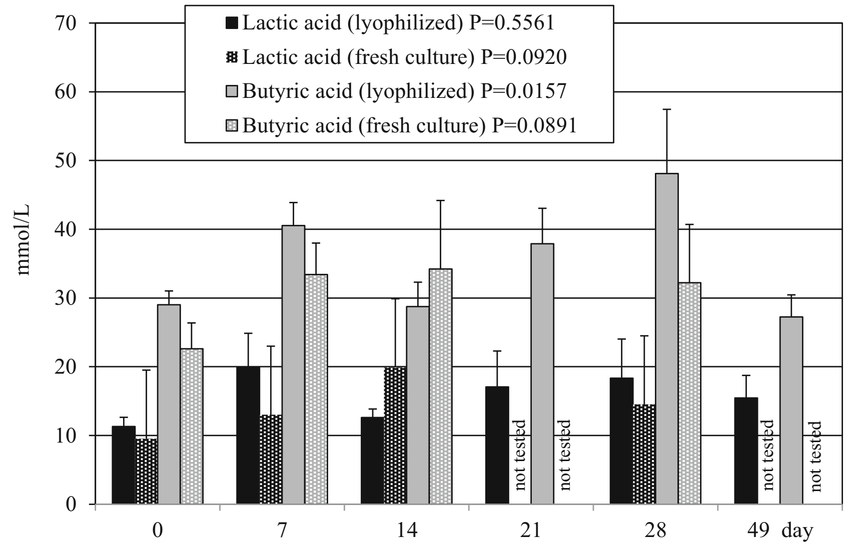

Fig. 1 The faecal concentration of lactic and butyric acid of the dogs supplemented with fresh or lyophilized form of *L. fermentum* CCM 7421 for 14 days

of D-lactic acid that becomes a concern. D-lactic acid is broken down much more slowly than L-lactic acid (mammals possess only L-lactic dehydrogenase), and when it begins to accumulate in the intestine it can push absorption into the blood. In patients with D-lactic acidosis, intestinal microbiota adapted in favour of Gram-positive anaerobes such as *Lactobacillus* species (Ku et al. 2006). The probiotic strain *L. fermentum* CCM 7421 produces a mixture of L- and D-lactic acids, and therefore we decided to test combinations with alkaline or pH-restoring additives such as chlorophyllin and alginite (Strompfová et al. 2015, Kubašová et al. 2016). In both studies, faecal pH was maintained (no decrease) whereas reduction of clostridia and coliform bacteria was still significant. This means that some antimicrobial effect was observed despite there being no decrease in pH value in the intestinal environment. Studies measuring L- and D-lactate in faecal content or blood serum remain to be conducted to see the complete effect of such additive combinations.

Effect on serum biochemistry

A desirable way of health monitoring is the study of clinical biochemistry in experimental animals. Although the levels of certain biochemical parameters in blood are often less specific or sensitive indicators of health status (there is still considerable ability of compensation by the healthy parts of tissue and organs, or through the metabolic bypaths), their measurement forms the data base for most diagnostic investigations.

Since changes in serum biochemistry do not appear in an immediate process, our experiments with the application of *L. fermentum* CCM 7421 were set up to collect blood samples over a 7-week long period (5 post-treatment weeks). Plasma proteins represent a heterogeneous group with albumin constituting the major portion (55%). Plasma and serum proteins act as anions in the acid-base balance, take part in coagulation reactions and serve as carriers for many compounds. The concentration of total protein remained stable in most of our canine experiments after application of CCM 7421 strain (using healthy dogs) although an increase in most dogs with hypoproteinaemia (suffering from acute or chronic gastrointestinal disorders) was detected after 7-day probiotic application (Strompfová et al. 2007, Table 2). Significant increase was also noted in healthy dogs with initial levels under 61 g/L (Strompfová et al. 2006). Albumin is synthesized in the liver just as all other plasma proteins except immunoglobulins (produced by lymphoid tissue), and is catabolised by all metabolically active tissues. It serves as a regulator of osmotic equilibrium. Its concentrations decrease during infection or inflammation processes, which has also been observed in dogs (Zapryanova et al. 2013). The level of albumin tested in several of our experiments increased by 1–1.5 g/L after 14-day application in healthy dogs (Kubašová et al. 2016; Strompfová et al. 2012b). Increase in protein fractions due to the presence of probiotic bacteria are often ascribed to increased efficiency of digestion (secretion of probiotic bacteria-derived proteases and peptidases) and nutrient absorption processes (e.g. increased villus height; Neis et al. 2015). This effect is important especially in dogs which are losing weight (e.g. due to malabsorption syndromes or malnutrition). In general, the most commonly tested biochemical parameters in serum are cholesterol and triglycerides. Although they are of importance in humans in relation to vascular diseases, dogs normally have an abundance of beneficial high-density lipoproteins which allow them to eat large quantities of animal fats (Maldonado et al. 2002). Cholesterol is the precursor of cholesterol ester, bile acids and steroid hormones; it is an ancillary aid in the diagnosis of several metabolic diseases (e.g. hypothyroidism, diabetes mellitus, hyperadrenocorticism). *L. fermentum* CCM 7421 has a regulatory effect on cholesterol levels detected in both healthy and

Table 2 The overview of effects observed after application of *L. fermentum* CCM 7421 alone or in combination with additive in dogs

Dosage	Used form	Length of application	Observed physiological effects (except microbiology)	Reference
10^8	Fresh	4 days[a]	↑ weight gains (by 14%); ↑lactic acid in small intestinal content; ↑ phagocytic activity of leukocytes (+index)	Strompfová et al. 2005
10^9	Fresh	7 days	↑ serum total protein and lipid; serum↓ glucose	Strompfová et al. 2006
10^9	Fresh	7 days	↓ serum alanine aminotransferase; ↑↓ regulatory effect on cholesterol and total protein; improvement of faecal consistency in dogs with diarrhoea	Strompfová et al. 2007
10^7	Lyophilized	7 days	↓ faecal pH (trend); ↓ serum glucose; ↓ serum alkaline phosphatase	Strompfová et al. 2012a
10^8	Fresh	14 days	↑ succinic acid in faeces, ↑ butyric and lactic (trend); ↓ faecal pH (trend); ↑ phagocytic activity of neutrophils and monocytes; ↑ respiratory burst of phagocytes (trend); ↑ haemoglobin concentration; ↑ serum glucose	Strompfová et al. 2015
10^8	Fresh	14 days	↓ faecal pH; ↑ phagocytic activity of leukocytes (+index); ↑ serum glucose	Strompfová et al. 2012b
10^8	Fresh	14 days	↓ feacal pH; ↑ serum glucose	Strompfová et al. 2013
10^8	Fresh	14 days	↓ faecal pH (trend); ↑ haemoglobin concentration; ↑ phagocytic activity of leukocytes; ↑ respiratory burst (trend); ↑ serum glucose; ↓ serum calcium; ↑ lactic and butyric in faeces (trend); ↑ succinic acid	Kubašová et al. 2016
10^8	Lyophilized	14 days	↓ faecal pH; ↑ formic, butyric, valeric, succinic acid in faeces; ↑ phagocytic activity of leukocytes (+index); ↑ serum albumin; ↑ serum glucose	Strompfová et al. 2012a
L. fermentum CCM 7421 + additive				
10^8	Fresh + inulin (1% of diet)	14 days	↓ faecal ammonia concentration; ↓ faecal pH	Strompfová et al. 2013
10^8	Fresh + E. senticosus (8 mg/kg BW)	14 days	↑ phagocytic activity of leukocytes; ↓ serum aspartate aminostransferase; more liquid faeces (higher faecal score)	Strompfová et al. 2012b
10^8	Fresh + chlorophyllin (60 mg/dog)	14 days	↑ lactic acid in faeces (trend); ↑ phagocytic activity of neutrophils and monocytes; ↑ respiratory burst	Strompfová et al. 2015
10^8	Fresh + alginite (1% of diet)	14 days	↑ haemoglobin concentration; ↓ serum cholesterol; ↑ serum magnesium; ↑ formic acid in faeces	Kubašová et al. 2016

[a] Tested in Japanese quails

ill dogs (Strompfová et al. 2006, 2007), whereby reduction of cholesterol values in dogs with higher levels and contrastingly an increase in lower levels within the physiological norm was detected (however with no significant changes in average values). Blood glucose, an important source of energy for many cells, is normally maintained by the breakdown of dietary carbohydrates and a complex of endogenous production (glycogenolysis and gluconeogenesis). The maintenance of normal plasma glucose requires delicate balance between glucose availability and utilisation. Disorders of the liver, pancreas and kidneys or alterations in glucoregulatory hormones (insulin, glucagon, cortisol, epinephrine) cause hypoglycaemia and hyperglycaemia. The most commonly observed effect of *L. fermentum* CCM 7421 was significant increase in glucose levels at the end of strain application and at 14-day post-treatment sampling (Kubašová et al. 2016; Strompfová et al., 2012b). The increase in glucose concentration can be explained by conversion of produced lactic acid into pyruvic acid and then to glucose through hepatic gluconeogenesis (Bongaerts et al. 2006). Alanine transaminase (ALT) plays an important role in gluconeogenesis by converting alanine into pyruvate for glucose production. Increased gluconeogenesis is therefore often connected with increased activity of this enzyme (Leena et al. 1999). In dogs, stable activity of serum ALT in healthy dogs (Strompfová et al. 2006, 2013), significant decrease in dogs suffering from acute gastrointestinal disease and slight decrease in dogs with chronic gastrointestinal syndrome was observed (Strompfová et al. 2007). This enzyme is present in high concentrations in the cytoplasm of hepatocytes and thus is specific for detection of liver cell damage, which could be associated with several inflammatory and infectious intestinal diseases. The last determinants in evaluating the profiles of dogs were serum minerals such as calcium, phosphorus or magnesium. They are important ions in the body, utilized in bone and structural organisation, enzyme function, blood coagulation and maintaining osmotic pressure of fluid balances, and they are essential in muscle activity. Inadequate nutrition and renal or parathyroid hormone disorders are among the most frequent causes of fluctuations in these minerals. Application of *L. fermentum* CCM 7421 did not disturb the concentration of phosphorus, while calcium levels were stable

(Strompfová et al. 2013) or significantly lower after 14-day application (Kubašová et al. 2016). This was a point that we dealt with in our combination experiments. Our aim was to achieve stability in the acid-base balance through combination with additives having no antimicrobial impact on the probiotic strain. The addition of chlorophyll as well as alginite prevented decrease in both minerals in blood serum during the experiments without negative disruption of other probiotic strain effects (Kubašová et al. 2016; Strompfová et al. 2015). Unfortunately, there is a lack of studies testing serum biochemistry in dogs, limiting the possibility of results comparison. Our study using canine-originated *Bifidobacterium animalis* B/12 revealed significantly lower concentrations of triglyceride and albumin and significantly higher levels of ALT and alkaline phosphatase using the same experimental scheme with a 14-day application period (Strompfová et al., 2014). Another canine study with *E. faecium* EE3 administered for 7 days showed reduction of total protein and lipids compared to initial values, and cholesterol was influenced to reach the reference range (Marciňáková et al. 2006). More probiotic studies testing their effects on serum biochemistry during and after treatment in dogs are necessary.

Effect on haematology

Detection of the haematological profile provides important information about the type, number and appearance of cells in the blood and is a valuable tool in the diagnosis and prognosis of many disorders such as anaemia, dehydratation, infection, myeloproliferative disorders or immunosuppression. Although the blood cell count remained stable in two experiments with application of *L. fermentum* CCM 7421 in dogs (Kubašová et al. 2016; Strompfová et al. 2015) as well as in Japanese quails (Strompfová et al. 2005), the concentration of haemoglobin was significantly higher (by 6 g/L) in canine experiments 2 weeks after probiotic ingestion ceased. The exact mechanism of this effect is still not known, although the enhancement of trace mineral absorption (e.g. iron) through the production of short-chain fatty acids (Bouglé et al. 2009) or increased production of vitamin B12 and folate (LeBlanc et al. 2010)—factors important for haemopoesis—are well documented. Similarly, significant increase in haemoglobin concentration but also in haematocrit, monocytes and neutrophils is described in the study by Baillon et al. (2004) in dogs consuming probiotic strain *L. acidophilus* DSM13241 for 4 weeks.

Effect on immunological parameters

One of most important ways in which probiotic lactic acid bacteria can provide physiological benefit is modulation of host immune response, either innate or specific. Probiotic bacteria have been shown to influence immune responses non-specifically by enhancing phagocytosis of pathogens and modifying cytokine production as well (Maldonado et al. 2015). Our studies with *L. fermentum* CCM 7421 demonstrated significant increases in total phagocytic activity of leukocytes and phagocytic activity of neutrophils at the end of the treatment period (14 days; Kubašová et al. 2016; Strompfová et al. 2015) or after several weeks of post-treatment period in dogs (Strompfová et al. 2012b). The same result was observed in Japanese quails after 3 days of post-treatment period (Strompfová et al. 2005). The mechanisms of this probiotic action are still not well understood, but upregulation of the expression of phagocytic receptors such as CR1, CR3, FcγRIII and FCαR in the neutrophils has been proposed (Pelto et al. 1998). Another tested parameter was oxidative burst activity, a crucial reaction that occurs in phagocytes to degrade internalized particles and bacteria. A trend towards increase in this activity (expressed as an index) has been noted in our canine experiments (Kubašová et al. 2016; Strompfová et al. 2015). Application of *B. animalis* B/12 in dogs for 2 weeks led similarly to significant increases in total leukocyte phagocytosis and phagocytic activity of neutrophils (Strompfová et al. 2014). Heat-killed *E. faecalis* FK-23 administered to healthy dogs in a single dose was also associated with increased neutrophil phagocytosis and cytokine production (Kanasugi et al. 1997). This indicates that this effect seems to be a more common characteristic following probiotic lactic acid bacteria consumption independently from viability. Studies involving other immune parameters are planned.

Conclusion

The strain *L. fermentum* CCM 7421 has been tested in nine independent studies either alone or in combination with other natural additives. The following commonly observed effects of the strain can be considered: increased lactic acid bacteria population during the application (maximum counts of applied strain in the first week of administration), prolonged reduction of clostridia population and partially of some Gram-negative bacterial genera and modulation of liquid faecal consistency to normal (dogs with diarrhoea). In blood, optimization of several biochemical parameters (total protein, cholesterol, ALT) and significant stimulation of non-specific cellular immunity parameters have been detected. It is necessary to mention that the scope and depth of response after application of CCM 7421 strain depend on the initial health status. Naturally, more significant changes have been detected in dogs requiring modulation of their microbiological, biochemical or immunological parameters. Studies using molecular approaches are planned to evaluate alterations in canine microbiome after application of *L. fermentum* CCM 7421 more complexly also during different medical conditions.

Compliance with ethical standards

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national and institutional giudelines for the care and use of animals were followed.

This article does not contain any studies involving human participants performed by any of the authors.

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