

## PROBIOTIC STRAIN *LACTOBACILLUS FERMENTUM* CCM 7421, CANINE ISOLATE APPLIED TO DOGS SUFFERING FROM GASTROINTESTINAL DISORDERS

Viola Strompfová<sup>1</sup>, Miroslava Marciňáková<sup>1</sup>, Monika Simonová<sup>1</sup>, Andrea Lauková<sup>1</sup>, and Mária Fialkovičová<sup>2</sup>

<sup>1</sup>Institute of Animal Physiology, Slovak Academy of Sciences, Šoltésovej 4-6, 040 01 Košice, Slovakia; <sup>2</sup>I. Internal Clinic, Department of Small Animals, University of Veterinary Medicine, Komenského 73, 040 01 Košice, Slovakia

**ABSTRACT:** Increasing tendency of gastrointestinal diseases occurrence in dogs lead to study of natural and safe ways to treat them. In the present study, preliminary effect of *Lactobacillus fermentum* CCM 7421 strain (our canine isolate) with probiotic properties to help in the treatment of 14 dogs with clinical symptoms indicating acute or chronic disorders of gastrointestinal tract was investigated. The strain CCM 7421 was applied once a day at a dose of 3 ml/dog (10<sup>8</sup> CFU/ml) for 7 days. The faeces and blood samples were collected before the beginning and after 7 days of application. After application, significant increase in the population of lactic acid bacteria - lactobacilli and enterococci in faeces ( $p < 0.01$ ) was determined and the counts of *Escherichia coli* in majority of dogs were reduced. Concerning the biochemical parameters, significant decrease of alanine aminotransferase in dogs with acute gastrointestinal diseases ( $p < 0.01$ ) was detected. On the other hand, an increase of total protein in dogs with hypoproteinemia was noted and regulative effect in cholesterol level, as well. Clinically, watery faeces was arranged to normal consistency in relatively short time in most of dogs. *L. fermentum* CCM 7421 seems to have beneficial effect in acceleration to recover from digestive disorders of dog; of course, further studies are necessary.

**KEY WORDS:** Blood, Dog, Faeces, Gastrointestinal disease, *Lactobacillus fermentum*, Probiotic

*Corresponding Author:* Dr. Viola Strompfová, Institute of Animal Physiology SAS, Šoltésovej 4-6, 040 01 Košice, Slovakia, Fax: +421-55-7287842; E-mail: [strompfv@saske.sk](mailto:strompfv@saske.sk)

### INTRODUCTION

The gastrointestinal tract (GIT) together with respiratory tract belong to the largest body surface areas; activity of the intestinal microbiota is believed to be comparable to that of the liver. In spite of its important role in health, the knowledge of the canine intestinal microbiota is scarce (Greetham et al., 2002; Strompfová et al., 2004; Štetina et al., 2005). In addition to the quantity of food/feed (chemicals including which passes through this canal, the GIT represent frequently disturbed

organ also in pets. Diarrhoea is a commonly encountered problem in small animal practice and a number of causes of diarrhoea are recognized (changes in food, bacterial, viral, fungal, protozoal, parasitic infections, toxins, antibiotics, foreign material, enzyme deficiency, neurogenic). One of the most common causes of diarrhoea is the ingestion of a food or substance not ordinarily consumed in the diet (T. J. Dunn, Jr. DVM, Director, ThePetCenter.com). Sudden changes in the dog diet (switching to a different brand of food/feed) can cause the imbalance within the GIT flora that is triggered by new substrates on which the GIT flora grow and is reproduced. However, an optimal balance in the GIT flora can disrupt many other factors such as antibiotic therapy, stress, environmental changes, pollutants, chemicals in agriculture, including age. All these factors can contribute to reduction of beneficial bacteria (lactic acid bacteria) relevant in the prevention of invasion by pathogens and allow overgrowth of species with potential pathogenicity (e.g. *Clostridium difficile*, Van der Waaij, 1989; Sullivan et al., 2001).

Correction of the properties of an unbalanced indigenous microbiota forms the rationale of probiotic therapy. Probiotics – preparations or products containing viable, defined microorganisms in sufficient numbers, which alter the microflora in a compartment of the host and thus exerting beneficial health effects in the host (Schrezenmeir and de Verse, 2001) – have been examined for their effectiveness in the prevention and treatment of various human gastrointestinal disorders and have been considered as successful in the cases of lactose intolerance, Crohn's disease, gastrointestinal infections (*Helicobacter pylori*, enterotoxigenic *E. coli*, *Shigella* sp., *Salmonella* sp., *Campylobacter* sp., *Clostridium difficile*, rotavirus), antibiotic-associated diarrhoea, travellers' diarrhoea, inflammatory bowel disease, food hypersensitivity and allergy, constipation (de Roos and Katan, 2000; Marteau et al., 2001; Erdeve, 2007). Many of them can occur also in dogs; however, studies documenting the therapeutic effect of probiotics in pets are rare (Holakovská, 2005). Various mechanisms by which probiotics could be of benefit for diarrhoea have been proposed (Rolfe, 2000; Görke and Liebler-Tenorio, 2001; Gionchetti et al., 2006). These include: reduction in gastrointestinal pH through stimulation of lactic acid-producing bacteria; a direct antagonistic action against gastrointestinal pathogens; competition with pathogens for binding and receptor sites;



### Isolation and enumeration of intestinal microflora

One g of faeces sample was mixed with 9 ml of sterile saline buffer (0.85 %, pH 7.0) and homogenized (3 min) using a stomacher (IUL, Instruments, Spain). Samples were serially diluted according to the standard microbiological method and 0.1 ml of appropriate dilution was plated onto the following media: Mac Conkey agar for enumeration of *Escherichia coli*, Mannitol salt agar for staphylococci, Baird-Parker agar supplemented with Egg Yolk Tellurite Solution for coagulase-positive staphylococci and M-Enterococcus agar for enterococci (all from Becton and Dickinson, Cockeysville, USA), Cetrimide agar (Biomark, India) for *Pseudomonas*-like bacteria, MRS agar (Merck) for lactobacilli, and MRS agar with rifampicin (100 µg/ml) for *L. fermentum* CCM 7421. They were cultivated at 37 °C for 24-48 h, lactobacilli were incubated in a 3 % CO<sub>2</sub> atmosphere and cultivated for 48-72 h. *Pseudomonas*-like bacteria were cultivated at 25 °C for 48-72 h. Numbers of CFU were expressed as log<sub>10</sub> CFU per gram. The results are given as arithmetical means ± standard deviation (SD).

### Analysis of biochemical parameters in blood

Analysis of biochemical parameters in blood was performed following: 30 min. after blood sampling, samples were centrifuged (3000 x g for 10 min.) and sera were tested spectrophotometrically by using of BIO-LA-TEST (Lachema, a.s., Czech Republic) for total protein (TP 300), total lipid (TL 180), cholesterol (CHOL 150) and alanine aminotransferase (ALT 360).

### Statistical analysis

Statistical evaluation of the results was performed by paired Student's *t*-test with the level of significance set at  $p < 0.05$ . Data obtained after 7 days application of the strain CCM 7421 were compared to control data – data achieved at day 0.

## RESULTS

### Biochemical parameters in blood

The concentration of total protein was slightly increased (by 5.7 g/l in dogs with acute GIT disease – no. 1-7 and by 2.3 g/l in dogs with GIT chronic disease – no. 8-14 in average, Table 1). The total protein levels were under the physiological level (57.0 - 75.0 g/l) in 6 dogs and they were increased in 4 dogs into physiological level after application of *L. fermentum* CCM 7421. The total lipid concentration was slightly increased (increase by 1.2 g/l in dogs with chronic disease) or remained stable in average (dogs with acute disease). Values of total lipid were over physiological level (4.70 - 7.25 g/l, Table 1) in 3 individuals and under level in 1 dog before application of CCM 7421 strain. They were arranged into physiological level in 2 dogs at the end of experiment. The strain CCM 7421 reduced or regulated serum cholesterol values in experimental dogs (reduction by 0.4 - 0.5 mmol/l in average in both groups of dogs). The regulating effect was visible in majority of dogs. It means, the CCM 7421 strain reduced cholesterol values in dogs with higher level and oppositely it increased the lower cholesterol levels in

the framework of physiological norm (2.60 - 6.47 mmol/l) as well as it decreased value over physiological level in 1 of 2 dogs. The concentration of ALT was significantly decreased in dogs suffering from acute GIT disease ( $p < 0.01$ ) and slightly decreased in dogs suffering from chronic disease (by 0.065 µkat/l in average).

### Faecal microflora

The results for the population of faecal microflora indicated that application of *L. fermentum* CCM 7421 was associated with considerable decrease of total count of *E. coli* in the faeces of most dogs (71 %, Table 2). In detail, numbers of *E. coli* decreased by 1.1 log<sub>10</sub> CFU/g in dogs no. 1-7 and by 0.7 log<sub>10</sub> CFU/g in dogs no. 8-14 in average. The number of enterococci significantly ( $p < 0.01$ ) increased after the strain administration in both groups of dogs (by 1.2 log<sub>10</sub> CFU/g in dogs no. 1-7 and by 2.1 log<sub>10</sub> CFU/g in dogs no. 8-14 in average, Table 2). There was visible effect of CCM 7421 strain application on faecal lactobacilli counts which were significantly increased ( $p < 0.01$ ; by 2.8 log<sub>10</sub> CFU/g in dogs no. 1-7 and by 2.8 log<sub>10</sub> CFU/g in dogs no. 8-14 in average). Total counts of CCM 7421 strain ranged between 7.8 and 9.7 log<sub>10</sub> CFU/g during its application. Numbers of coagulase negative as well as coagulase-positive staphylococci and *Pseudomonas*-like bacteria in the faeces of dogs were not unambiguously influenced. Although, they were decreased in most of dogs included in experiment (57 - 64 %, Table 2).

TABLE 2. Total counts of selected bacterial groups in faeces of ill dogs (dogs with acute disease no. 1-7; dogs with chronic disease no. 8-14) before and after application of strain

| Dog            | Day | <i>E. coli</i> | <i>Ent.</i> | <i>Lact.</i> | CCM 7421 | CPS        | <i>Staph.</i> | <i>Pseud.</i> |
|----------------|-----|----------------|-------------|--------------|----------|------------|---------------|---------------|
| 1              | 0   | 4.6            | 5.3         | 5.7          | -        | 3.0        | 3.4           | 0.1           |
|                | 7   | 5.4            | 7.4         | 8.8          | 8.2      | 4.2        | 3.0           | 2.9           |
| 2              | 0   | 4.2            | 7.9         | 8.0          | -        | 2.1        | 4.3           | 1.2           |
|                | 7   | 4.4            | 8.5         | 9.5          | 8.0      | 1.2        | 3.8           | 0.6           |
| 3              | 0   | 5.0            | 5.3         | 6.2          | -        | 2.1        | 4.2           | 2.0           |
|                | 7   | 2.7            | 6.4         | 9.6          | 8.1      | 1.3        | 4.4           | 0.1           |
| 4              | 0   | 7.6            | 6.0         | 8.0          | -        | 1.0        | 3.0           | 2.4           |
|                | 7   | 6.6            | 6.2         | 9.3          | 8.6      | 2.3        | 1.7           | 4.0           |
| 5              | 0   | 8.1            | 8.3         | 3.9          | -        | 1.0        | 1.6           | 1.0           |
|                | 7   | 7.0            | 9.8         | 9.3          | 8.3      | 0.6        | 1.4           | 0.1           |
| 6              | 0   | 9.3            | 7.7         | 3.7          | -        | 2.3        | 1.3           | 2.3           |
|                | 7   | 5.9            | 8.4         | 8.0          | 8.4      | 2.7        | 2.1           | 2.6           |
| 7              | 0   | 6.4            | 5.0         | 6.8          | -        | 1.9        | 1.4           | 3.4           |
|                | 7   | 5.3            | 7.0         | 7.5          | 8.2      | 1.0        | 1.8           | 2.5           |
| <i>t</i> -test |     | $p > 0.05$     | $p < 0.01$  | $p < 0.01$   |          | $p > 0.05$ | $p > 0.05$    | $p > 0.05$    |
| 8              | 0   | 5.0            | 3.6         | 8.8          | -        | 1.4        | 1.0           | 3.1           |
|                | 7   | 4.7            | 6.9         | 8.9          | 7.8      | 1.0        | 1.7           | 3.1           |
| 9              | 0   | 8.2            | 3.1         | 3.0          | -        | 2.4        | 2.0           | 2.6           |
|                | 7   | 5.0            | 6.2         | 8.7          | 8.3      | 0.1        | 3.2           | 4.7           |
| 10             | 0   | 8.0            | 8.1         | 7.9          | -        | 3.4        | 4.3           | 3.7           |
|                | 7   | 7.5            | 9.0         | 10.4         | 9.1      | 1.0        | 2.0           | 3.1           |
| 11             | 0   | 11.4           | 6.2         | 6.8          | -        | 1.4        | 2.3           | 1.7           |
|                | 7   | 8.2            | 9.7         | 9.4          | 9.7      | 1.0        | 1.9           | 1.4           |
| 12             | 0   | 3.3            | 6.6         | 5.9          | -        | 0.9        | 1.5           | 1.6           |
|                | 7   | 6.2            | 7.8         | 9.2          | 7.8      | 1.5        | 3.9           | 1.2           |
| 13             | 0   | 6.5            | 7.4         | 5.3          | -        | 2.3        | 3.8           | 1.9           |
|                | 7   | 7.3            | 8.6         | 9.4          | 8.0      | 1.2        | 1.8           | 0.1           |
| 14             | 0   | 7.8            | 7.7         | 7.8          | -        | 1.3        | 2.1           | 3.2           |
|                | 7   | 6.3            | 9.0         | 9.0          | 8.4      | 1.7        | 1.9           | 3.2           |
| <i>t</i> -test |     | $p > 0.05$     | $p < 0.01$  | $p < 0.01$   |          | $p > 0.05$ | $p > 0.05$    | $p > 0.05$    |

*Ent.*: Enterococcus sp.; *Lact.*: Lactobacillus sp.; CPS: coagulase-positive staphylococci; *Staph.*: Staphylococcus sp.; *Pseud.*: Pseudomonas-like bacteria



### Further observations

Clinically, changes of faeces consistency to normal was detected during 3-5 days in dogs having watery faeces before application experiment (no. 2, 4, 6, 9, 11, 12, 13). In faeces of dog no. 9, no oocysts of *Cystoisospora* sp. (initial: +) after CCM 7421 application were found. Owners of dogs stated an increase of appetite in most of experimental dogs (78%). No clinically evident adverse effects were observed during administration of the strain CCM 7421.

### DISCUSSION

The strain of *L. fermentum* CCM 7421 was well accepted by ill dogs. As concerned biochemical parameters, tendency of probiotic strains to increase serum protein level (Bomba et al., 1997; Lorek et al., 2001) was also indicated (although slightly, not significantly) in this experiment. However, that increase was less visible in comparison with the results after CCM 7421 strain application in healthy dogs (Strompfová et al., 2006). Although, positive result was arrangement - increase of its level in several dogs with hypoproteinemia. The same result reported Holakovská (2005) after application of *L. rhamnosus* GG to healthy dogs. Increase of total protein level via better utilisation and absorption of proteins in feed is important result especially in dogs losing weight (e.g. malabsorptive disorders, malnutrition). Recently, it was shown that probiotics might induce the formation of short chain fatty acids and vitamins and thus contribute to the colonic trophism (Sakata et al., 1999) that could be one of the factors enhancing nutrient absorption following increase of body weight. Although, our experiment lasted short time to detect changes in body weight of dogs, *L. fermentum* CCM 7421 administered to 2-days-old Japanese quails caused increase of daily weight gain by 14 % (Strompfová et al., 2005). Similarly, the concentration of total lipid was influenced; however, not so visibly than in healthy dogs. Reduction in the increase of serum total protein and lipid in ill dogs comparing with healthy dogs was probably due to inflammation of intestinal tract altering absorption of nutrients from intestine. Regulating effect of CCM 7421 strain on cholesterol level in blood was detected even in previous experiment with healthy dogs (Strompfová et al., 2006). Similarly, application of *Enterococcus faecium* EE3 and *L. rhamnosus* GG to healthy dogs caused regulation of cholesterol level in the framework of physiological limit (Holakovská, 2005; Marciňáková et al., 2006). Necessity of this effect is lower since in dogs there are twice as much HDL than LDL, and 80 % of the total cholesterol is binded to HDL. Alanine aminotransferase is principally found in the liver and is regarded as specific for detecting liver cell damage (Johnston, 1999). Hepatocytes play a major role in absorbing and metabolising many toxic chemicals. They are therefore liable to injury by various chemicals, including food. Application of the strain CCM 7421 to ill dogs was associated without negative influence on hepatocytes, rather the opposite, it decreased level in some dogs with higher level of ALT. The lower ALT values in rats treated with *L. acidophilus* or *L. casei* alone compared to the control detected also Oyetayo et al. (2003). In addition, experiment of Adawi et al. (2001) in an acute liver injury rat model showed that administration of different lactobacilli reduced bacterial

translocation and hepatocellular damage including significant reduction of the levels of ALT.

The first idea with probiotics in past was always to change the composition of the normal intestinal microflora from a potentially harmful composition towards a microflora that would be beneficial for the host. In accordance with this idea, administration of *L. fermentum* CCM 7421 to ill dogs lead to considerable increase of lactic acid bacteria (lactobacilli, enterococci) and decrease of *E. coli* in majority of dogs. Equal results were obtained in previous experiments in Japanese quail and healthy dogs (Strompfová et al., 2005; Strompfová et al., 2006). Marciňáková et al. (2006) reported decrease of faecal enterococci, but increase of lactobacilli after *E. faecium* EE3 application for 7 days in healthy dogs. Consumption of *L. salivarius* UCC118 caused increase in faecal *Enterococcus* levels (Mattila-Sandholm et al. 1999) as in our experiment. Reduction of *E. coli* could be caused by lactic acid production by CCM 7421 strain since a significantly higher concentration of lactic acid in small intestinal contents of Japanese quails after 4 days application of this strain was previously detected by Strompfová et al. (2005). There is hypothesis that volatile fatty acids produced by probiotic bacteria posses potential bactericidal activity and that the bactericidal activity of the organic acids depends mainly on their undissociated form which can permeate the cell membrane by diffusion and release protons in the cell. The influx of protons is thought to induce acidification of the cytoplasm and dissipate the membrane proton potential (Brocklehurst and Lund, 1990). Certainly, more other mechanisms could contribute to decrease numbers of *Enterobacteriaceae*.

Clinically, watery faeces in dogs was arranged to normal consistency in relatively short time. However, more studies demonstrated shortening of diarrhoea duration (Tláškal et al., 2007), the mechanism of this antidiarrhoeal effect is not described in detail. Although, several mechanisms has been indicated in certain types of diarrhoea in human trials such as digestion of toxins by protease secreted by probiotic *Saccharomyces boulardii* in the case of diarrhoea and colitis caused by *Clostridium difficile* (Castagliuolo et al., 1999) or increase levels of circulating immunoglobulin IgA by *L. casei* in infants infected with rotavirus (Perdigon et al., 1991). Additionally, the normalization of increased intestinal permeability during rotavirus infection or Crohn's disease has also been reported (Isolauri, 2004).

In conclusion, the present results indicated that the 7 days lasting application of canine isolate - *L. fermentum* CCM 7421 to dogs with gastrointestinal disorders at least positively altered intestinal microflora (higher numbers of lactobacilli, enterococci; reduction of *E. coli*) and increased total protein in dogs with hypoproteinemia, as well as regulated cholesterol level. Although, our preliminary study was limited concerning the animal counts, it may be helpful when planning continuation or similar studies with dogs. The involvement of higher numbers of dogs and detection of other parameters would enable a more definite verification of the observed effects.

### ACKNOWLEDGEMENTS

This study was supported by the project VEGA 2/5139/27 of Slovak Scientific Agency. We are grateful for the assistance



of Dr. Peter Smrčo and dogs' owners in realization of experiment as well as for excellent technical assistance of Margita Bodnárová.

## REFERENCES

- Adawi, D., Ahrne, S., and Molin, G. (2001) Effects of different probiotic strains of *Lactobacillus* and *Bifidobacterium* on bacterial translocation and liver injury in an acute liver injury model. *International Journal of Food Microbiology* 70: 213-220.
- Bomba, A., Gancarčíková, S., Nemcová, R., Herich, R., Čížek, M., and Kapitančík, B. (1997) Aplikácia *Lactobacillus casei* v kŕmárstve s rozvinutou črevnou mikrobiotou (In Slovak). *Animal Production* 42: 355-360.
- Brocklehurst, T. F., and Lund, B.M. (1990) The influence of pH, temperature and organic acids on the initiation of growth of *Yersinia enterocolitica*. *Journal of Applied Bacteriology* 69: 390-397.
- Castagliuolo, I., Riegler, M.F., Valenick, L., LaMont, J.T., and Pothoulakis, C. (1999) *Saccharomyces boulardii* protease inhibits the effects of *Clostridium difficile* toxins A and B in human colonic mucosa. *Infection and Immunity* 67: 302-307.
- De Roos, N.M., and Katan, M.B. (2000) Effects of probiotic bacteria on diarrhea, lipid metabolism, and carcinogenesis: a review of papers published between 1988 and 1998. *The American Journal of Clinical Nutrition* 71: 405-411.
- Erdeve, O. (2007) Prophylactic use of probiotics for prevention of antibiotic-associated diarrhea in children. *International Journal of Probiotics and Prebiotics* 2: 9-14.
- Gionchetti, P., Rizzello, F., Morselli, C., Tambasco, R., Calabrese, C., Campieri, M. (2006) Probiotics and prebiotics in inflammatory bowel disease: existing evidence and potential benefits. *International Journal of Probiotics and Prebiotics* 1: 97-104.
- Görke, B., and Liebler-Tenorio, E. (2001) Probiotika: Gibt es wissenschaftliche Grundlagen für ihre Wirkung? *Deutsche Tierärztliche Wochenschrift* 108: 233-280.
- Greetham, H.L., Giffard, C., Hutson, R.A., Collins, M.D., and Gibson, G.R. (2002) Bacteriology of the Labrador dog gut: a cultural and genotypic approach. *Journal of Applied Microbiology* 93: 640-646.
- Holakovská, K. (2005) Testování vlivu a stability probiotického kmene *Lactobacillus rhamnosus* GG v trávicím traktu psů (in Czech), PhD thesis, University of Veterinary Medicine, Košice, Slovakia.
- Isolauri, E. (2004) The role of probiotics in pediatrics. *Current Paediatrics* 14: 104-109.
- Johnston, D.E. (1999) Special considerations in interpreting liver function tests. *The American Academy of Family Physicians*. April 15, 1999.
- Lorek, O.M., Gugolek, A., and Hartman, A. (2001) Nutrient digestibility and nitrogen retention in arctic foxes fed a diet containing cultures of probiotic bacteria. *Czech Journal of Animal Science* 46: 485-488.
- Marcináková, M., Simonová, M., Stropfová, V., and Lauková, A. (2006) Application of *Enterococcus faecium* EE3 in healthy dogs. *Folia Microbiologica* 51: 239-242.
- Marteau, P., de Vrese, M., Cellier, C.J., and Schrezenmeir, J. (2001) Protection from gastrointestinal diseases with the use of probiotics. *American Journal of Clinical Nutrition* 73: 430S-436S.
- Mattila-Sandholm, T., Blum, S., Collins, J.K., Crittenden, R., de Vos, W., Dunne, C., Fondén, R., Grenov, G., Isolauri, E., Kiely, B., Marteau, P., Morelli, L., Ouwehand, A., Reniero, R., Saarela, M., Salminen, S., Saxelin, M., Schiffrin, E., Shanahan, F., Vaughan, E., and von Wright, A. (1999) Probiotics: towards demonstrating efficacy. *Trends in Food Science and Technology* 10: 393-399.
- Oyetayo, V.O., Adetuyi, F.C., and Akinyosoye, F.A. (2003) Safety and protective effect of *Lactobacillus acidophilus* and *Lactobacillus casei* used as probiotic agent *in vivo*. *African Journal of Biotechnology* 2: 448-452.
- Perdigon, G., Alvarez, S., and Pesce de Ruiz Holgado, A. (1991) Immuno-adjunct activity of oral *Lactobacillus casei*: influence of dose on the secretory immune response and protective capacity in intestinal infections. *Journal of Dairy Research* 58: 485-496.
- Rolfe, R.D. (2000) The role of probiotic cultures in the control of gastrointestinal health. *The Journal of Nutrition* 130: 396S-402S.
- Sakata, T., Kojima, T., Fujieda, M., Miyakozawa, M., Takahashi, M., and Ushida, K. (1999) Probiotic preparation dose-dependently increase net production rates of organic acids and decrease that of ammonia by pig cecal bacteria in batch culture. *Digestive Diseases and Sciences* 44: 1485-1493.
- Schrezenmeir, J., and de Vrese, M. (2001) Probiotics, prebiotics, and synbiotics – approaching a definition. *The American Journal of Clinical Nutrition* 73: 361S-364S.
- Stropfová, V., Lauková, A., and Ouwehand, A.C. (2004) Lactobacilli and enterococci – potential probiotics for dogs. *Folia Microbiologica* 49: 203-207.
- Stropfová, V., Marcináková, M., Gancarčíková, S., Jonecová, Z., Sciranková, J., Guba, P., Koščová, J., Boldiřarová, K., and Lauková, A. (2005) New probiotic strain *Lactobacillus fermentum* AD1 and its effect in Japanese quail. *Veterinary Medicine-Czech* 50: 415-420.
- Stropfová, V., Marcináková, M., Simonová, M., Bogovic-Matijašić, B., and Lauková, A. (2006) Application of potential probiotic *Lactobacillus fermentum* AD1 strain in healthy dogs. *Anaerobe* 12: 75-79.

## 6 Probiotic lactobacilli and gastrointestinal disorders

Sullivan, A., Edlund, C., and Nord, C.E. (2001) Effect of antimicrobial agents on the ecological balance of human microflora. *The Lancet Infectious Diseases* 1: 101-114.

Štětina, V., Lauková, A., Strompfová, V., Švec, P., and Sedláček, I. (2005) Identification of *Staphylococcus piscifermentans* from fog faeces. *Folia Microbiologica* 50: 524-528.

Tláškal, P., Kokešová, A., Schramlová, J., Tlaskalová-Hogenová, H., Adamus, J., Bubáková, D., Kocnarová, N., Kopecký, K., Mucková, M., Pacovská, J., and Sládková, J. (2007) *Lactobacillus acidophilus*, *Lactobacillus reuteri* and metabolites of intestinal bacteria as therapeutic agents in acute diarrhea in children. *International Journal of Probiotics and Prebiotics* 2: 67-74.

Van der Waaij, D. (1989) The ecology of the human intestine and its consequences for overgrowth by pathogens such as *Clostridium difficile*. *Annual Review of Microbiology* 43: 69-87.