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Feed supplementation of *Lactobacillus plantarum* PCA 236 modulates gut microbiota and milk fatty acid composition in dairy goats — a preliminary study

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ABSTRACT

This study aimed to evaluate the potential of a promising *Lactobacillus plantarum* isolate (PCA 236) from cheese as a probiotic feed supplement in lactating goats. The ability of *L. plantarum* to survive transit through the goat gastrointestinal tract and to modulate selected constituents of the gut microbiota composition, monitored at faecal level was assessed. In addition, *L. plantarum* effects on plasma immunoglobulins and antioxidant capacity of the animals as well as on the milk fatty acid composition were determined. For the purpose of the experiment a field study was designed, involving 24 dairy goats of the Damascus breed, kept in a sheep and goat dairy farm. The goats were divided in terms of body weight in two treatments of 12 goats each, namely: control (CON) without addition of *L. plantarum* and probiotic (PRO) treatment with in feed administration of *L. plantarum* so that the goats would intake 12 log CFU/day. The experiment lasted 5 weeks and at weekly time intervals individual faecal, blood and milk samples were collected and analysed. All faecal samples were examined for the presence of *L. plantarum* PCA 236. In addition, the culturable population levels of mesophilic aerobes, coliforms lactic acid bacteria (LAB), *Streptococcus*, *Enterococcus*, mesophilic anaerobes, *Clostridium* and *Bacteroides* in faeces were also determined by enumeration on specific culture media. In parallel, plasma IgA, IgM and IgG and antioxidant capacity of plasma and milk were determined. No adverse effects were observed in the animals receiving the lactobacillus during the experiment. *Lactobacillus plantarum PCA 236* was recovered in the faeces of all animals in the PRO treatment. In addition, PRO treatment resulted in a significant ($P \leq 0.05$) increase in LAB coupled with a significant decrease in faecal clostridia populations compared to the CON treatment. The antioxidant capacity and the concentrations of immunoglobulins IgA, IgM and IgG in goat plasma did not differ between the treatments. In contrast, milk fat composition in the PRO treatment had a significantly higher content of polyunsaturated fatty acids such as linoleic, a-linolenic and rumenic acids compared to CON, while there were no differences in milk antioxidant capacity. The results obtained in this study, indicate that the *L. plantarum* PCA 236 strain has displayed an interesting probiotic potential, in terms of beneficially modulating the goat faecal microbiota and milk fatty acid composition that needs to be further researched.

1. Introduction

In recent years, research on lactic acid bacteria (LAB) has been escalating concerning their beneficial effects on human and animal health as probiotics (Fuller, 1989; Guarner and Schaafsma, 1998), beyond their desirable properties in food technology and as protective cultures (Holzapfel et al., 1995). In humans, there is growing evidence that probiotic intake could result in improved intestinal condition and health, improved host nutrition and beneficial modulation of the immune system (Salminen et al., 1998; Rowland, 1999; Saarela et al., 2002; Rastall et al., 2005). Probiotic applications in animal nutrition aim to promote production performance and prevention of diseases via the maintenance of a healthy gastrointestinal environment and improved intestinal function (Patterson and Burkholder, 2003; Chaucheyras-Durand et al., 2008; Mountzouris et al., 2009).

There are several reports in the literature on the use of various bacteria and yeasts as probiotics in farm animals. In monogastric

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animals, most of the research has focused on pigs and poultry. The species examined belong mainly to bacteria such as *Bacillus*, *Enterococcus* and *Lactobacillus* or various combinations of more than one bacterium (Patterson and Burkholder, 2003; Mountzouris, 2006; Scharek et al., 2007; Casey et al., 2007; Mountzouris et al., 2007; Tsuda et al., 2008). On the other hand, there are far less studies researching probiotics, also known as "direct fed microbials" in ruminant nutrition. Contrary to monogastric animals, most of the research on probiotic applications in ruminants has focused in yeasts (Stella et al., 2007; Chaucheyras-Durand et al., 2008). However, the application of LAB as well as mixtures containing LAB in cows and calves date back several years (Gomez-Alarcon et al., 1991; Abe et al., 1995). In sheep, Asby et al. (1989) and Birch et al. (1994) were among the first to research the use of probiotic bacteria as feed supplements. More recently, feed supplementation with *E. faecium* and a mixture of lactobacilli reduced *E. coli* 0157:H7 faecal counts and improved meat production in lambs (Lema et al., 2001), while a probiotic mixture of yeasts, LAB and bacilli administered in ewes resulted in changing the faecal composition with respect to mould and bacilli concentration (Kumagai et al., 2004). The administration of *B. licheniformis* and *B. subtilis* in ewes had a beneficial effect on milk yields as well as milk fat and protein content (Kritas et al., 2006). Even less information is currently available for probiotic research in goats. The administration of fermented milk curd with live LAB in goats led to a decrease in diarrhoea incidence and an increase in weight gain (Anandan et al., 1999) as well as an improved metabolic profile (Chiofalo et al., 2004).

Recently *Lactobacillus plantarum* PCA 236 (ACA-DC 201) strain was isolated from naturally fermented Kasseri cheese. So far, the strain has been found to display interesting properties *in vitro* that could render it as a candidate probiotic. Such properties include survival in conditions mimicking the gastrointestinal tract (low pH, bile salts), production of bacteriocin (plantaricin EF), and absence of haemolytic activity or antibiotic resistance against commonly used antibiotics (unpublished data). In addition, the particular strain produced a protective effect against viral disruption of goat intestinal epithelium cell lines (Maragkoudakis et al., 2010).

It was therefore speculated that the particular *L. plantarum* strain of dairy origin could be an interesting candidate probiotic for dairy animals. Due to the high importance of goat and sheep husbandry towards the economics of the overall animal production of the countries around the Mediterranean basin, goats were chosen for the purpose of this work. The aim of this work was to investigate the effects of *L. plantarum* administration as a probiotic feed supplement in dairy goats. For that reason, the survival of the strain through the animal's gastrointestinal tract, known to be a desirable probiotic property (FAO/WHO, 2002), was examined. In addition, in order to gain an insight of potential probiotic function on gut microbiota and host level, the effects of *L. plantarum* administration on selected bacteria of the faecal microbiota composition, goat plasma immunoglobulins and antioxidant status as well as on milk fatty acid composition were also investigated.

2. Materials and methods

2.1. Animals

Twenty four female goats of the Damascus breed were used in this study. The experiment was performed in a dairy goat farm in Nicosia from the middle of May till the end of June 2008. All animals were well within their lactation period as their parturitions had occurred between February and March of that year and were housed indoors with access to an exercise area. Animals were machine milked twice per day (i.e. morning and evening) in the dedicated milking room facility of the farm. During the experiment all animals continued the nutritional scheme practiced by the farm. In particular, goat nutrition was based on the intake of forage consisting of oat hay and barley straw offered *ad libitum* and 1 kg of concentrate. The concentrate consisted of (g/kg): maize grain, 223; barley grain, 270; soybean meal, 180; dried lucerne, 140; sunflower meal, 100; wheat bran 60; limestone, 15; calcium phosphate 5; salt, 5; and mineral and vitamin premix, 2. Each animal received the concentrate feed in two equal portions (i.e. 500 g) during the morning and evening milking procedure.

2.2. Experimental treatments and probiotic administration

The animals were divided in two equal groups ($n=12$) of similar body weight (60 ± 1.7 kg). Each group of animals was assigned to one of the following two treatments: control (CON, with no probiotic) or probiotic (PRO) with probiotic administration. The probiotic strain *L. plantarum* PCA 236 (ACA-DC 201, Culture collection of the Agricultural University of Athens, Greece), isolated from Kasseri cheese, was used as a lyophilised powder preparation (Probiotical S.p.a., Italy) that had a viable count of 11.4 log CFU/g and was stored at 4 °C for the duration of the experiment. *Lactobacillus plantarum* was administered twice daily, during the morning and evening milking time, for a period of 35 days. In particular, 2 g of lyophilized *L. plantarum* were added by top dressing the 500 g of concentrate feed mix offered individually per milking time, for a total administration of 12.0 log CFU/goat per day. During the experiment, animals belonging to different treatments were housed and milked separately in order to prevent direct contact and interaction.

2.3. Faecal microbiota analysis

Freshly voided faecal samples were collected from every animal in both groups during the evening milking at days 0, 7, 14, 21 and 35 of the experiment. Upon collection, faecal samples from each animal ($n=12$ per treatment) were quickly sealed into 50×70 cm plastic bags filled with three anaerobic catalysts (Anaerocult A, Merck, Germany). The sealed bags were immediately placed into a portable refrigerator filled with ice blocks and sent to the laboratory for analysis. A representative 10 g faecal sample per animal ($n=12$) was homogenised in Maximum Recovery Diluent (MRD, Oxoid), using a stomacher laboratory blender (Seward, U.K). Enumeration of the faecal microbiota was carried out using the standard serial dilution method in maximum recovery diluent (MRD) on the appropriate selective media (Oxoid, UK). In particular, Mesophilic aerobic bacteria and coliforms were determined on Nutrient Agar and on MacConkey Agar No. 3, respectively, after 24 h of aerobic incubation at 37 °C. Mesophilic anaerobic bacteria were determined on Wilkins–Chalgren anaerobe agar (WC), Bacteroides on WC supplemented with a supplement selective for Gram negative anaerobes (G-N anaerobe selective supplement, Oxoid, UK) and defibrinated horse blood (Oxoid, UK), while Clostridia were enumerated on Reinforced Clostridial Agar supplemented with 20 μg/ml polymyxin (Sigma-Aldrich), all after 48 h incubation at 37 °C under anaerobic conditions using the Anaerocult A system (Merck, Germany). Lactic acid bacteria (LAB) were enumerated on de Man, Rogosa and Sharpe (MRS) agar, enterococci on Kanamycin Aesculin Azide Agar (KAA) supplemented with 20 μg/ml Kanamycin and streptococci on Columbia Blood Agar Base supplemented with a supplement selective for *Streptococcus* (Oxoid, UK), all incubated for 48 h in a 10% CO_2 incubator.

2.4. Detection of L. plantarum PCA 236 in faecal samples

Single colonies isolated from both MRS and the chromogenic *L. plantarum* Specific Medium (LPSM, Bujalance et al., 2006) agar plates with identical macroscopic and microscopic morphology with that of *L. plantarum* PCA236, were used for DNA extraction in a rapid lysis colony-PCR method (Veyrat et al., 1999). The *L. plantarum* species-specific PCR was carried out using the primers planF and pREV, according to Torriani et al. (2001). To confirm the identification results and to rule out false positives due to the presence of