

Antimicrobial activity of lactic acid bacteria multiplied in an alternative substrate and their influence on physiological parameters of new-born calves

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ABSTRACT: Here, the ability of *Pediococcus pentosaceus* and *Pediococcus acidilactici* to utilise potato tuber juice for cell synthesis without an external nutrient supplement was investigated, and the influence of lactic acid bacteria (LAB) grown in this substrate on the growth performance of new-born calves, as well as blood biochemical and faecal microbiological parameters was evaluated. Calves were selected based on the analogy principle, treatment group ($n = 21$), control group ($n = 27$). Calves in the treatment group were administered 50 ml of fermented potato tubers juice containing 9.6 log CFU/ml of LAB mixture for 14 days. Also, determination of antimicrobial activities of tested LAB against a variety of pathogenic and opportunistic bacterial strains previously isolated from diseased cattle was performed. It was found that LAB supernatants effectively inhibited the growth of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enterica*, *Corynebacter* spp., *Klebsiella pneumoniae*, *Enterococcus faecalis*, and *Bacillus cereus* (the diameters of the inhibition zones varied between 11.0 ± 0.3 mm and 17.0 ± 0.6 mm). Thus, potato juice can be used as an alternative substrate for LAB cultivation (LAB cell concentration 9.6 ± 0.07 log CFU/ml). After lyophilisation (-48 °C) and spray-drying ($+150$ °C) viable cell concentrations in the fermented potato juice powder were 9.18 ± 0.09 log CFU/g and 9.04 ± 0.07 log CFU/g, respectively. The 50 ml of fermented potato tuber juice containing 9.6 log CFU/ml of LAB, administered every day for 14 days, reduced the risk of developing acidosis (stabilised blood pH; $P < 0.05$), reduced lactates and PCO₂ concentration ($P < 0.05$) and the risk of liver lesions (reduced serum alanine aminotransferase concentration; $P < 0.005$) in blood and *E. coli* in the faeces of new-born calves.

Keywords: alternative substrate; antimicrobial activity; blood parameters; calves; faecal parameters; lactic acid bacteria

In calves less than 10 days old, infection commonly causes severe diarrhoea and high mortality (Busconi et al. 2008). Antibiotic usage for prevention of infections in animals should be avoided; thus, the possibility of using lactic acid bacteria (LAB) as an alternative treatment is promising (Bujnakova et al. 2014).

Over the last years, interest in bacteriocin-like inhibitory substances (BLIS) producing LAB has been increasing because of their potential use as

natural antimicrobial agents to enhance the safety of feed. The spontaneous fermentation is mainly the result of the proliferation of the microbial population contained in the raw materials. This microbiota consists mostly of LAB and yeasts but it might also contain coliforms, *Salmonella* and moulds. During the fermentation process, LAB converts sugars primarily into lactic acid and acetic acid. Consequently, the pH of the medium decreases and the environment becomes hostile to contaminants

such as *Enterobacteriaceae* (Plumed-Ferrer and von Wright 2009).

Often, LAB strains are incubated in an expensive commercially available de Man Rogosa Sharpe medium; the cultures are then centrifuged and the cells are washed with sterile water. This suspension can be used as a starter culture (Tsuda et al. 2012). Potato juice is an industrial waste which may constitute a source of digestible nutrients for microorganisms (Bzducha-Wrobel et al. 2014). Due to its low cost and good chemical composition (Al-Weshahy and Rao 2009), potato juice could have potential application in the feed industry.

During fermentation, microorganisms produce organic acids, which reduce the pH of the feed to 3.5–4.5 (Olstorpe et al. 2010). In addition, L-(+) can be safely used for feed. However, the potential toxicity of D-(-)-lactic acid is a concern, and thus, research is needed with regard to the content of D-(-)-lactic acid in fermented feed.

Calves are susceptible to many pathogens which can affect their subsequent performance. The health of heifers under 90 days of age has been shown to have long-term impacts on their future productivity (Stanton 2014). The use of (LAB) has been identified as a tool to maintain the intestinal microbial balance and to prevent the establishment of opportunistic pathogenic bacterial populations (Signorini et al. 2012). However, a consensus has not been reached as to whether probiotics may be effective in reducing the incidence of disease in young calves.

In our study the ability of LAB to utilise potato tuber juice for cell synthesis without an external nutrient supplement was investigated, and the influence of multiplied LAB on calf growth performance, blood biochemical and faecal microbiological parameters was evaluated. Also, determination of antimicrobial activities of the tested LAB against a variety of pathogenic and opportunistic bacterial strains previously isolated from diseased cattle was performed.

MATERIAL AND METHODS

Lactic acid bacteria. The LAB strains *Pediococcus acidilactici* and *Pediococcus pentosaceus* previously isolated from spontaneous fermented rye (SME Baltijos Biotechnologijos, Kaunas, Lithuania) were stored at $-80\text{ }^{\circ}\text{C}$ and cultured at $32\text{ }^{\circ}\text{C}$, for 48 h

in MRS broth (CM0359, Oxoid Ltd, Basingstoke, UK) with the addition of 40 mmol/l fructose and 20 mmol/l maltose prior to use.

Evaluation of antimicrobial activities of *P. acidilactici* and *P. pentosaceus*. The LAB were grown in de Man Rogosa Sharpe (MRS) medium (Biolife, Italy) at their optimal temperatures of $32\text{ }^{\circ}\text{C}$ (*P. acidilactici*), or $35\text{ }^{\circ}\text{C}$ (*P. pentosaceus*). Two percent of LAB cells were then inoculated into fresh medium and incubated for 18 h. The cells were harvested by centrifugation (6 000 g, 10 min, $4\text{ }^{\circ}\text{C}$). The culture supernatants were filtered through a 0.2 mm sterile Millipore filter to remove all cells. Supernatants were used for the determination of antimicrobial activities of *P. acidilactici* and *P. pentosaceus* strains against a variety of pathogenic and opportunistic bacterial strains (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enterica*, *Corynebacter* spp., *Klebsiella pneumoniae*, *Enterococcus faecalis*, and *Bacillus cereus*) previously isolated from diseased cattle. The agar well diffusion assay was used for testing of antimicrobial activities. For this purpose, 0.5 McFarland Unit density suspensions of each pathogenic bacterial strain were inoculated onto the surface of cooled Mueller Hinton Agar (Oxoid Ltd, Basingstoke, UK) using sterile cotton swabs. Wells of 6 mm in diameter were punched in agar and filled with 50 μl of LAB supernatants. The experiments were repeated three times and the average size of inhibition zones was calculated.

The antimicrobial activities against tested bacteria were determined by measuring the diameter of inhibition zones (mm).

Lactic acid bacteria multiplication and stabilisation in alternative substrate. Pure LAB were multiplied in a crushed potatoe juice medium. Before use, potato juice was sterilised. No additives were added to the juice. The sterilised potato juice, and LAB cell suspensions (5 ml), containing 8.9 log colony-forming units (CFU) per ml of *P. acidilactici* and *P. pentosaceus* were used to multiply the LAB (*P. acidilactici* at $32\text{ }^{\circ}\text{C}$, *P. pentosaceus* at $35\text{ }^{\circ}\text{C}$) for 24, 48 and 72 h. The final colony number in the potato juice was on average 9.60 log CFU/g. LAB that had multiplied in the alternative substrate were mixed (50/50; v/v) and used to feed calves. Analyses of fermented potato juice were conducted on *P. acidilactici* and *P. pentosaceus* mixtures (after 24, 48 and 72 h of fermentation).

In order to stabilise the LAB, two methods of dehydration were tested: lyophilisation (Milrock

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Kieffer Lane, Kingston, USA) and dehydration in a spray-drying system (SD-06, Keison, Great Britain). LAB that had multiplied in crushed potato juice medium were injected into the spray-drying system in a peristaltic fashion (inlet temperature +60 °C, inlet air temperature +150 °C, outgoing air temperature +80 °C, air flow 200 m³/h). The LAB CFU/g in the spray-dried and lyophilised powder was determined. The stability of dehydrated LAB during the storage (after four, eight and 12 months) was also investigated.

Determination of pH, total titratable acidity and L-(+)/D-(-)-lactic acid isomers of fermented potato juice. The pH value was measured and recorded using a pH electrode (PP-15, Sartorius, Goettingen, Germany). Total titratable acidity (TTA) was determined on 10 ml of sample homogenised with 90 ml of distilled water and expressed as the amount (in ml) of 0.1M NaOH needed to achieve a pH of 8.2. The concentrations of L-(+)- and D-(-)-lactic acid were determined using an enzyme test kit (R-biopharm AG-Roche, Darmstadt, Germany), as reported elsewhere (De Lima et al. 2009).

Design of the calf feeding experiment. A total number of 48 Holstein female calves were randomly divided into two homogeneous groups on the basis of body weight from the second day of life: control group, fed with a standard milk replacer diet, and treated group, fed with the same diet supplemented with 50 ml of fermented potato juice containing 9.6 log CFU/ml of LAB (*P. acidilactici* and *P. pentosaceus* mixture). Fermented potato juice was added to the morning milk feeding. Each calf was placed in an individual outdoor box (2.00 × 1.25 m), with free access to water. Calves were fed individually once a day (07:00 h), with non-medicated milk replacer (22.5% crude protein, 18% fat, 9.0% ashes, 1.75% lysine, 0.55% methionine and 0.50% cysteine on a dry matter basis). The milk powder (130 g/l) was reconstituted in hot water (65 °C) and fed at a temperature of 39 °C in a bucket. The initial amount of milk replacer was 4 l/calf/day. On Day 2 and 14 of life individual samples of blood and faeces were collected. Moreover, on Day 14 of life body weight was determined.

Analysis of blood parameters of calves. Blood samples of 5 ml were taken from the jugular vein into vacuum blood tubes (BD Vacutiner, United Kingdom) at two and 14 days of calf life. Tubes containing lithium heparin were used to study blood gas, and the tubes with clot activator were

used for biochemical examination of blood. The blood gas test was analysed using a blood gas analyser (EPOC, Canada): pH; PCO₂; PO₂; Na; K; iCa; glucose; lactates; haematocrit; HCO₃; TCO₂; sO₂; haemoglobin. The parameters were calculated automatically. After collection of blood into the vacutainer tubes with clot activator samples were centrifuged at 6000 g for 10 min to obtain plasma (Hettich Universal, UK) and serum was evaluated. The levels of serum alanine aminotransferase (AST) were measured using a Hitachi 705 (Hitachi, Japan) automated blood chemistry analyser and DIAS (Diagnostic Systems GmbH, Germany) reagents.

Faecal collection and enumeration of LAB and *Escherichia coli*. Individual faecal samples were collected on Day 2 and 14 of life by rectal stimulation, stored in vials (+4 °C) with transport medium (FaecalTM enteric Plus, Oxoid Ltd, Basingstoke, UK), and analysed on the same day. Ten grams of each sample were diluted with 90 ml of Buffered Peptone Water (Oxoid Ltd, Basingstoke, UK) and homogenised in a Stomacher for 1 min (Seward Stomacher 400 blender Mixed Homogenizer, International PBI, Milano, Italy). 10-fold serial dilutions were spread using sterile spatula onto MRS agar medium (Oxoid Ltd, Basingstoke, UK) as reported by Ripamonti et al. (2009) and TBX agar (ISO 16649-2) (Oxoid Ltd, Basingstoke, UK) for the enumeration of LAB and *E. coli*, respectively. MRS agar plates were incubated in anaerobic jars (Anaerobar, Oxoid Ltd, Basingstoke, UK) with the Anaerogen kit (Oxoid Ltd, Basingstoke, UK) at 37 °C for 48 h, while TBX agar plates were incubated aerobically at 37 °C for 24 h. After incubation, the agar plates were assessed for growth and typical colonies were counted.

Growth performance. Individual body weight was recorded at two and 14 days of age, using an electronic weighing system (model BF/E 1425E, Technosystem, Italy).

Statistical analysis. All the experiments of LAB antimicrobial activities and analytical determinations of fermented potato juice results were performed at least in two independent replicates. The means and standard deviations of the data were calculated.

All analytical determinations of calf blood parameters were performed in triplicate. Obtained data were analysed using the statistical package SPSS for Windows XP V15.0 (SPSS Inc., Chicago, USA, 2007). The significance of differences between treated samples was evaluated using the paired *t*-test.

Table 1. Inhibition of the growth of pathogenic bacteria by lactic acid bacteria

Microorganisms	Zone of inhibition/mm*	
	<i>P. acidilactici</i>	<i>P. pentosaceus</i>
<i>P. aeruginosa</i>	15 ± 0.5	17 ± 0.6
<i>S. aureus</i>	16 ± 0.6	16 ± 0.5
<i>E. coli</i>	15 ± 0.3	13 ± 0.3
<i>S. enterica</i>	9 ± 0.4	11 ± 0.3
<i>Corynebacterium</i> spp.	11 ± 0.3	12 ± 0.4
<i>K. pneumoniae</i>	11 ± 0.3	11 ± 0.6
<i>E. faecalis</i>	14 ± 0.5	13 ± 0.3
<i>B. cereus</i>	11 ± 0.3	14 ± 0.5

*The diameter of wells was 6 mm

RESULTS

Antimicrobial activities of *P. acidilactici* and *P. pentosaceus*

Antimicrobial activities of LAB are presented in Table 1. As can be seen from the obtained results LAB supernatants inhibited the growth of all tested bacteria. The diameters of the inhibition zones toward pathogenic strains varied between 11.0 ± 0.3 mm and 17.0 ± 0.6 mm. The highest antimicrobial activity was demonstrated by *P. pentosaceus* against *Pseudomonas aeruginosa* (inhibition zone diameter was 17.0 ± 0.6 mm). The lowest inhibition zones were observed against *S. enterica* (9 ± 0.4 mm by *P. acidilactici* and 11 ± 0.3 mm by *P. pentosaceus* supernatants) and *K. pneumoniae* (11 ± 0.3 mm; 11 ± 0.3 and 11 ± 0.6 mm by *P. acidilactici* and *P. pentosaceus*, respectively).

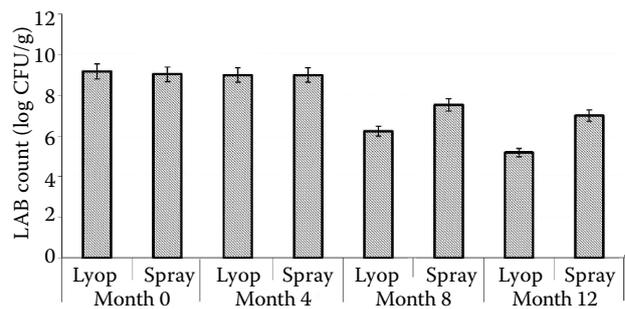


Figure 1. Lactic acid bacteria (LAB) count (log CFU/g) of fermented and dehydrated potato juice powder after 0, 4, 8 and 12 months of storage

Lyop = lyophilised, Spray = spray dried

Lactic acid bacteria multiplication and stabilisation in potato juice substrate

Results of pH, TTA, dry matter, L-(+)-/D(-)-lactic acid isomer content and cell growth observed at 0, 24, 48 and 72 h of fermentation in potato juice media are presented in Table 2. Acid concentration increased through fermentation, and after 72 h of fermentation the pH of the samples was 4.1 ± 0.01 and the TTA $8.92 \pm 0.06^\circ\text{N}$. It was found that the *P. acidilactici* and *P. pentosaceus* strains used in potato juice produced a very small amount of D-lactic acid, and that the major isomer was L-lactic acid. At 72 h the viable cell concentration in the fermented potato juice was 9.6 ± 0.07 log CFU/ml. Also, it was found that after lyophilisation (-48°C) and spray-drying ($+150^\circ\text{C}$) the viable cell concentration in the fermented powder products was 9.18 ± 0.09 log CFU/g and 9.04 ± 0.07 log CFU/g,

Table 2. pH, total titratable acidity (TTA), L(+)-/D(-)-lactic acid isomer content, dry matter (dm) and lactic acid bacteria (LAB) counts of fermented potato juice and LAB counts of dehydrated products, data are presented as mean ± SD ($n = 3$)

Fermentation time	pH	TTA ($^\circ\text{N}$)	D(-) (g/100g)	L(+) (g/100g)	dm (%)	LAB (log CFU/ml) in potato juice	LAB (log CFU/g) powder obtained by	
							lyophilisation	spray-drying
0 h	5.85 ± 0.01	2.31 ± 0.04	–	–	3.3 ± 0.01	–	9.18 ± 0.09	9.04 ± 0.07
24 h	4.52 ± 0.01	8.04 ± 0.03	0.07 ± 0.02	1.73 ± 0.05	3.1 ± 0.01	9.04 ± 0.01		
48 h	4.39 ± 0.02	8.28 ± 0.07	0.09 ± 0.03	1.92 ± 0.03	3.0 ± 0.01	9.07 ± 0.04		
72 h	4.10 ± 0.01	8.92 ± 0.06	0.14 ± 0.01	2.10 ± 0.07	3.0 ± 0.01	9.60 ± 0.07		
P-value	0.0012	0.0207	0.0407	0.0031	< 0.0001	0.0004		

Paired *t*-test, column statistics

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Table 3. Calf blood parameters

Parameters	Treatment group		Control group		P
	Day 2	Day 14	Day 2	Day 14	
pH	7.36	7.36	7.33	7.28	**
PCO ₂ (mmHg)	63.95	70.9	63.08	60.71	**
Lactate (mmol/l)	3.20	2.64	3.95	4.29	**
PO ₂ (mmHg)	20.20	36.26	21.48	38.88	n.s.
Na (mmol/l)	136.38	136.13	137.17	136.67	n.s.
K (mmol/l)	5.19	4.64	5.03	5.38	n.s.
ICa (mmol/l)	1.30	1.31	1.30	1.31	n.s.
Glucose (mmol/l)	6.06	5.74	7.43	4.95	n.s.
Haematocrit fraction	0.28	0.27	0.27	0.24	n.s.
HCO ₃ (mmol/l)	35.38	34.16	33.73	34.03	n.s.
TCO ₂ (mmol/l)	37.31	36.01	35.70	36.22	n.s.
O ₂ saturation	0.29	0.52	0.30	0.63	n.s.
Haemoglobin (g/l)	96	92.8	93.3	83	n.s.
AST (μkat/l)	0.84694	0.56270	0.85306	1.0013	***

n.s. = not significant

P* < 0.01, *P* < 0.001

respectively (Table 2). Results showed, that after the storage (after 12 months) at room temperature (22 ± 2 °C) LAB counts in dehydrated products were 5.18 log CFU/g and 7.00 log CFU/g (in spray-dried and lyophilised powder, respectively; Figure 1). According to the obtained results, potato juice can be used as an alternative substrate for *P. acidilactici* and *P. pentosaceus* cultivation. Also, drying enables the use of the obtained powders as LAB starters on farms.

Calf blood parameters

Before the experiment, the blood pH of treatment group calves was 7.33, and in the control group the value was 7.36, whereas, after 14 days, the values had changed to 7.28 and 7.36, respectively (Table 3). Calf blood pH in the treatment group remained stable over the entire experimental period. The concentration of PCO₂ in the blood of control group calves increased from 63.95 to 70.93, while in the treatment group this value decreased from 63.08 to 60.71. The concentration of lactate in the treatment group decreased from 3.20 mmol/l to 2.64 mmol/l, whereas, in the control group this value increased from 3.95 mmol/l to 4.29 mmol/l. The concentration of AST in the control group calves increased from 0.85306 μkat/l to 1.0013 μkat/l, whereas, in the treatment group this value decreased from 0.84694 μkat/l

to 0.56270 μkat/l. Significant differences between the other studied blood parameters were not identified.

Collection of faeces and enumeration of lactic acid bacteria (LAB) and *E. coli*

The results of the faecal *E. coli* counts demonstrated that calves receiving *P. acidilactici* and *P. pentosaceus* mixtures had significantly lower levels of *E. coli* in faeces on Day 14 compared with the control treatment, and significant higher LAB counts (Figure 2).

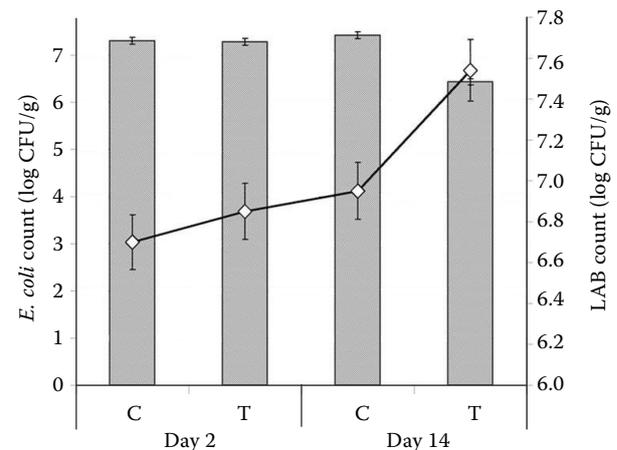


Figure 2. Numbers of lactic acid bacteria (◇ LAB) and *Escherichia coli* (■ log CFU/g of wet digesta) in calf faeces fed with or without supplement, *P* ≤ 0.05

C = control group, T = treated group

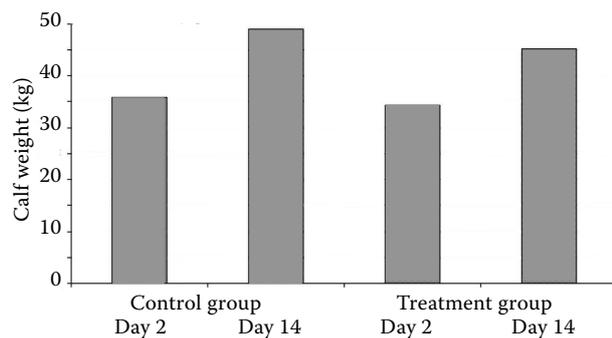


Figure 3. Calf weight (kg) after two and 14 days

Growth performance

Data on growth performance indicated that potato juice fermented with *P. acidilactici* and *P. pentosaceus* mixture does not have a significant influence on calf body weight (Figure 3). It was found that the body weight of control group calves increased from 35.75 kg to 49.00 kg, and that of the treatment group – from 34.37 to 45.25 kg.

DISCUSSION

Calf diarrhoea is a major cause of economic losses and is associated with high morbidity and mortality in the cattle industry worldwide (Bartels et al. 2010). Historically, calf diarrhoea has been commonly attributed to bovine rotavirus group A, bovine coronavirus, bovine viral diarrhoea virus, *Salmonella* spp., *E. coli*, *Clostridium perfringens* type C and *Cryptosporidium parvum*. Therefore, *Salmonella enterica* is commonly detected in faeces from diarrhoeic calves (Cho et al. 2013). In our experiments LAB inhibited the growth of both Gram-positive and Gram-negative bacteria including *Salmonella enterica* and *E. coli*. Antibacterial treatment plays a predominant role in the management of infectious diseases (Ozkanlar et al. 2012). The high frequency of co-infection by multiple pathogens in young animals emphasises the fact that interventions for calf diarrhoea should be focused on husbandry and management strategies, including assurance of colostrum intake, hygiene, reduction of population density, or modified components of the calving system (Larson and Tyler 2005). *Pediococcus* strains demonstrated good inhibition properties against all tested undesirable microorganisms *in vitro*, and could be used as ad-

ditives for the prevention of infectious diseases in calves, particularly after successful clinical trials performed *in vivo*.

Utilisation of cheap carbon sources from renewable resources is now considered as an effective approach in lactic acid production (Silveira et al. 2012). Moreover, it is necessary to minimise production costs in order to satisfy the current market demand for feed production. Agro-industrial products or residues represent the cheaper alternative substrates for industrial processes. The ability of LAB to use various sugars to produce the preservative lactic acid plays an important role in feed fermentation. Several factors affect the microbial and nutritional characteristics of the final product and therefore, an understanding of the impact of these factors on the characteristics of the mixture is crucial. The initial hours of incubation are characterised by high pH, low numbers of LAB and yeasts, high numbers of *Enterobacteriaceae*, and low concentrations of lactic acid, whereas at later hours of incubation, the pH and *Enterobacteriaceae* counts decrease, whereas the number of LAB and yeasts, as well as the concentration of organic acids and ethanol increase (Canibe and Borg 2012).

After 72 h of fermentation the concentration of viable *P. acidilactici* and *P. pentosaceus* cells in the fermented potato juice was 9.6 ± 0.07 log CFU/ml. This concentration is much higher in comparison with the concentration of viable LAB cells (*L. acidophilus* and *L. plantarum*) in a different composition of cereal medium (6 log CFU/ml; Rathore et al. 2012). Also, after lyophilisation (-48 °C) and spray-drying ($+150$ °C) the viable cell concentration in the fermented powder products was 9.18 ± 0.09 log CFU/g and 9.04 ± 0.07 log CFU/g, respectively. The unique properties of *P. acidilactici* DQ2 consist in its excellent thermo- and inhibitor-tolerance. It was demonstrated that under typical autoclave temperatures, after 30 min at 115 °C or 1 h 121 °C, *P. acidilactici* DQ2 still survived (Zhao et al. 2013). Fermentation was observed to decrease the pH and increase the TTA of the fermented potato juice, and the major isomer was L-lactic acid. According to Zhao et al. (2013), the L-lactic acid in the lactic acid produced by *P. acidilactici* DQ2 constituted 63.4%, while the D-lactic acid constituted 36.6%. This indicates that the products of *P. acidilactici* DQ2 fermentation were hetero-lactic acids, with approximately 2/3 being formed by L-lactic acid and 1/3 by D-lactic acid.

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Pediococcus species produce organic acids, hydrogen peroxide, and bacteriocins resulting in a pH decrease and inhibition of spoilage microorganisms, and thus contribute to the control of microbial proliferation during fermentation (Albano et al. 2007). Some strains of *Pediococcus* species produce antimicrobial peptides that inhibit closely related LAB as well as Gram-positive spoilage and pathogenic bacteria (Cizeikiene et al. 2013). Generally, inhibitory compounds are most likely secondary LAB metabolites which are produced after 48 h of fermentation (Rouse and van Sinderen 2008). Also, the unique thermo-tolerant properties of *P. acidilactici* may be of benefit for fermentation at high temperatures, which matches the optimal cellulase activity (50 °C or higher) and reduces the risk of microbial contamination during feed stock production. The antimicrobial peptide produced by *P. pentosaceus* was identified as a pediocin or a class IIa bacteriocin, which is a heat and cold-stable peptide with inhibitory activity against several Gram-positive pathogens (Papagianni and Papamichael 2014). According to our results, potato juice could be used as an alternative substrate for *P. acidilactici* and *P. pentosaceus* cultivation.

The generally recommended minimum levels of probiotics are 10^6 – 10^7 CFU/g or ml of product. However, probiotics tend not to survive well in high-moisture matrices (Liu and Tsao 2010). It was found that after 12 months of storage, LAB dehydrated by spray-drying survived in higher amounts in comparison with lyophilised (LAB count after 12 months of storage in lyophilised powder 5.18 log CFU/g, in spray-dried powder 7.53 log CFU/g). These results indicate that spray-drying could be a useful technique for the production of LAB starter, because LAB processed in this way is stable during storage.

Haematological and biochemical analyses of blood are of great use in obtaining insights into the metabolic and health status of an animal. Addition of *Bacillus licheniformis*, *Bacillus subtilis* and *Lactobacillus plantarum* to cow diet had no effect on serum biochemical parameters of animals (Fu et al. 2012). Administering probiotic (*Lactobacillus animalis* SB310, *Lactobacillus paracasei* subsp. *paracasei* SB137 and *Bacillus coagulans* SB117 in a 30:35:35 ratio, respectively; 1.8×10^{10} CFU/g of powder) during the first month of life improved gut microbiota and increased the growth performance and some biometric parameters of calves (Agazzi

et al. 2014). We found that fermented potato juice containing 9.6 log CFU/ml of LAB reduces AST concentrations ($P < 0.001$) in the blood of calves. The AST enzyme is found in different tissues and is a sensitive indicator of soft tissue damage. High AST activity is also observed in the liver and in the case of liver damage AST activity in serum increases. It is known that after first colostrum intake the AST activity in serum increases by 23 IU/l to 38 IU/l at the age of 3 h, which is most likely due to absorption from colostrum or because of activation of enzymes in the calf intestine as a consequence of colostrum intake (Kurz and Willet 1991). However, Hammon and Blum (1998) established that in calves which received only milk replacer instead of colostrum, the activity of AST increased on the second day after birth. Thus, these authors concluded that also other factors were responsible for the increased activity of certain enzymes. The activity of AST decreases after the first week, and from the 42nd to 84th day of life it increases slowly (Egli and Blum 1998). Mohri et al. (2007) observed an increase in AST activity from the 14th to the 84th day of age.

The acid-base balance and blood gases play an important role in the evaluation of metabolism in calves. Due to environmental and bodily factors, these parameters exhibit frequent deviations with marked effects on the health status of animals. A tendency to acidosis dominates, and alkalosis is quite rare. An understanding of the physiology underlying the growth and development of calves can frequently help in the clarification of various pathological states occurring at this stage (Nagy et al. 2003). It was found that 14-day administration of 50 ml of fermented potato juice containing 9.6 log CFU/ml of LAB per day, reduced the risk of developing acidosis and stabilised blood pH ($P < 0.05$) in calves.

Before weaning, dairy calves are susceptible to many pathogens and nutritional problems. For several years, antibiotics have been used to overcome these problems and also to promote economic benefits in terms of improved calf performance and reduced medication costs (Roodposhti and Dabiri 2012). The results of faecal *E. coli* counts illustrate that calves receiving *P. acidilactici* and *P. pentosaceus* mixtures had significantly lower levels of *E. coli* in faeces on Day 14 compared with the control treatment. There are two proposed mechanisms by which probiotics may reduce the levels of harmful

bacteria such as *E. coli* in the intestinal tract and therefore in faeces. Firstly, probiotic microorganisms produce some inhibitory substances such as organic acids, hydrogen peroxide and bacteriocins; these antimicrobial-like compounds might be active against some pathogens. Secondly, competitive inhibition by probiotic bacteria prevents adhesion of harmful bacteria on intestinal epithelial surfaces. Michael and Abney (2001) observed no significant differences in faecal *E. coli* populations between control calves and calves receiving probiotics and prebiotics. The use of LAB has been identified as a tool to maintain the intestinal microbial balance and to prevent the establishment of populations of opportunistic pathogenic bacteria (Signorini et al. 2012). Faecal counts of *Lactobacillus* are normally higher than counts of coliforms in healthy calves (LAB/coliforms ratio > 1) but, in calves suffering from diarrhoea, this relationship can change dramatically (Abu-Tarboush et al. 1996). Agazzi et al. (2014) demonstrated that the administration of LAB during the first month of life improved gut microbiota and increased the growth performance and some biometric parameters of calves. Gut microbial balance is one of the most important factors in determining good health status in young animals, particularly calves, where the immature immune system is prone to debilitating diarrhoea and respiratory diseases (Tsuruta et al. 2009). A growing number of studies in farm animals have reported that probiotic administration increases the number of beneficial bacteria and decreases the load of pathogens (Corcionivoschi et al. 2010; Signorini et al. 2012), thus positively affecting the function of the animal gut, and ensuring a lower incidence of intestinal and respiratory diseases. Oral administration of LAB-based probiotics has already been recognised as a promoter of intestinal microbial balance and growth performance (Nagashima et al. 2010), a role which it performs by promoting transient proliferation in the digestive tract as well as development of microbial defence against the growth of pathogenic bacteria.

We conclude that potato juice can be used as an alternative substrate for *P. acidilactici* and *P. pentosaceus* cultivation (after 72 h of fermentation the pH of the samples was 4.1 ± 0.01 ; TTA $8.92 \pm 0.06^\circ\text{N}$; LAB cell concentration $9.6 \pm 0.07 \log \text{CFU/ml}$). *P. acidilactici* and *P. pentosaceus* in potato juice produced a very small amount of D-lactic acid, and the major isomer was L-lactic acid. After drying

processed powders can be used on farms, and as LAB starters for feed fermentation. After lyophilisation (-48°C) and spray-drying ($+150^\circ\text{C}$) viable cell concentrations in the fermented powder products were $9.18 \pm 0.09 \log \text{CFU/g}$ and $9.04 \pm 0.07 \log \text{CFU/g}$, respectively. LAB supernatants effectively inhibited the growth of Gram-positive and Gram-negative pathogenic bacteria (the diameters of the inhibition zones varied between $11.0 \pm 0.3 \text{ mm}$ and $17.0 \pm 0.6 \text{ mm}$); therefore, both *Pediococcus* strains might be good candidates for further clinical trials aimed at the development of probiotic preparations. The 50 ml of fermented potato juice containing $9.6 \log \text{CFU/ml}$ of LAB per day, administered for 14 days, reduced the risk of developing acidosis (it stabilised blood pH; $P < 0.05$), reduced lactate and PCO_2 concentrations ($P < 0.05$), and the risk of liver lesions (reduced AST concentration; $P < 0.005$) in blood and *E. coli* in the faeces of calves.

REFERENCES

- Abu-Tarboush HM, Al-Saiady MY, Keir El-Din AH (1996): Evaluation of diet containing Lactobacilli on performance, fecal coliform and lactobacilli of young dairy calves. *Animal Feed Science and Technology* 57, 39–49.
- Agazzi A, Tirloni E, Stella S, Marocco S, Ripamonti B, Bersani C, Caputo JM, Dell'Orto V, Rota N, Savoini G (2014): Effects of species-specific probiotic addition to milk replacer on calf health and performance during the first month of life. *Annals of Animal Science* 14, 101–115.
- Al-Weshahy A, Rao V (2009): Isolation and characterization of functional components from peel samples of six potatoes varieties growing in Ontario. *Food Research International* 42, 1062–1066.
- Albano HM, Oliveira R, Arso N, Cubero T, Hogg P (2007): Antilisterial activity of lactic acid bacteria isolated from "Alheira" (traditional Portuguese fermented sausages). *In situ* assays. *Meat Science* 76, 796–800.
- Bartels CJ, Holzhauser M, Jorritsma R, Swart WA, Lam TJ (2010): Prevalence, prediction and risk factors of enteropathogens in normal and non-normal faeces of young Dutch dairy calves. *Preventive Veterinary Medicine* 93, 162–169.
- Bujnakova D, Strakova E, Kmet V (2014): In vitro evaluation of the safety and probiotic properties of Lactobacilli isolated from chicken and calves. *Anaerobe* 29, 118–127.
- Busconi M, Reggi S, Fogher EC (2008): Evaluation of biodiversity of lactic acid bacteria microbiota in the calf intestinal tracts. *Antonie van Leeuwenhoek* 94, 145–155.

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- Bzducha-Wrobel A, Blazejak S, Molenda M, Reczek L (2014): Biosynthesis of β (1, 3)/(1, 6)-glucans of cell wall of the yeast *Candida utilis* ATCC 9950 strains in the culture media supplemented with deproteinated potato juice water and glycerol. *European Food Research and Technology*, 1–12.
- Canibe N, Borg JB (2012): Fermented liquid feed – Microbial and nutritional aspects and impact on enteric diseases in pigs. *Animal Feed Science and Technology* 173, 17–40.
- Cho YI, Han JI, Wang C, Cooper V, Schwartz K, Engelken T, Yoon KJ (2013): Case-control study of microbiological etiology associated with calf diarrhea. *Veterinary Microbiology* 166, 375–385.
- Cizeikiene D, Juodeikiene G, Paskevicius A, Bartkiene E (2013): Antimicrobial activity of lactic acid bacteria against pathogenic and spoilage microorganisms isolated from food and their control in wheat bread. *Food Control* 31, 539–545.
- Corcionivoschi N, Drinceanu D, Pop I, Stack M, Stef D, Julean L (2010): The effect of probiotics on animal health. *Journal of Animal Science and Biotechnology* 43, 35–41.
- De Lima CJB, Coelho LE, Blanco KC, Contiero J (2009): Response surface optimization of D-(–)-lactic acid production by *Lactobacillus* SMI8 using corn steep liquor and yeast autolysate as an alternative nitrogen source. *African Journal of Biotechnology* 8, 5842–5846.
- Egli CP, Blum JW (1998): Clinical, hematological, metabolic and endocrine traits during the first three months of life of suckling simmentaler calves held in a cow-calf operation. *Journal of Veterinary Medicine: Series A* 45, 99–118.
- Fu Y, Diao Q, Tu Y, Wang J, Xu X (2012): Effects of different combinations of probiotics on growth performance and serum biochemical parameters in dairy calves aged from 0 to 8 weeks. *Chinese Journal of Animal Nutrition* 4. Available: http://en.cnki.com.cn/Article_en/CJFDTDOTAL-DWYX201204022.html.
- Hammon HM, Blum JW (1998): Metabolic and endocrine traits of neonatal calves are influenced by feeding colostrum for different durations or only milk replacer. *The Journal of Nutrition* 128, 624–632.
- Kurz MM, Willett LB (1991): Physiology and management; carbohydrate, enzyme, and hematology dynamics in newborn calves. *Journal of Dairy Science* 74, 2109–2118.
- Larson RL, Tyler JW (2005): Reducing calf losses in beef herds. *Veterinary Clinics of North America: Food Animal Practice* 21, 569–584.
- Liu SQ, Tsao M (2010): Enhancing stability of lactic acid bacteria and probiotics by *Williopsis saturnus* var. *saturnus* in fermented milks. *Nutrition and Food Science* 40, 314–322.
- Michael D, Abney BS (2001): Effects of feeding direct-fed microbials and prebiotics on receiving calf performance, health, and fecal shedding of pathogens. [MSc Thesis.] Texas Tech University, USA.
- Mohri M, Sharifi K, Eidi S (2007): Hematology and serum biochemistry of Holstein dairy calves: Age related changes and comparison with blood composition in adults. *Research in Veterinary Science* 83, 30–39.
- Nagashima K, Yasokawa D, Abe K, Nakagawa R, Kitamura T, Miura T, Kogawa S (2010): Effect of a *Lactobacillus* species on incidence of diarrhea in calves and change of the microflora associated with growth. *Bioscience of Microbiota* 29, 97–110.
- Nagy O, Seidel H, Kovac G, Paulikova I (2003): Acid-base balance and blood gases in calves in relation to age and nutrition. *Czech Journal of Animal Science* 48, 61–68.
- Olstorpe M, Axelsson L, Schnurer J, Passoth V (2010): Effect of starter culture inoculation on feed hygiene and microbial population development in fermented pig feed composed of a cereal grain mix with wet wheat distillers' grain. *Journal of Applied Microbiology* 108, 129–138.
- Ozkanlar Y, Aktas MS, Kaynar O, Ozkanlar S, Kirecci E, Yildiz L (2012): Bovine respiratory disease in naturally infected calves: clinical signs, blood gases and cytokine response. *Revue de Medecine Veterinaire* 163, 123–130.
- Papagianni M, Papamichael EM (2014): Production of pediocin SM-1 by *Pediococcus pentosaceus* Mees 1934 in fed-batch fermentation: Effects of sucrose concentration in a complex medium and process modeling. *Process Biochemistry* 49, 2044–2048.
- Plumed-Ferrer C, von Wright A (2009): Fermented pig liquid feed: nutritional, safety and regulatory aspects. *Journal of Applied Microbiology* 106, 351–368.
- Rathore S, Salmeron I, Pandiella SS (2012): Production of potentially probiotic beverages using single and mixed cereal substrates fermented with lactic acid bacteria cultures. *Food Microbiology* 30, 239–244.
- Ripamonti B, Agazzi A, Baldi A, Balzaretto C, Bersani C, Pirani S, Rebucci R, Savoini G, Stella S, Stenico A, Domeneghini C (2009): Administration of *Bacillus coagulans* in calves: recovery from fecal samples and evaluation of functional aspects of spores. *Veterinary Research Communications* 33, 991–1001.
- Roodposhti PM, Dabiri N (2012): Effects of probiotic and prebiotic on average daily gain, fecal shedding of *Escherichia coli*, and immune system status in newborn female calves. *Asian Australasian Journal of Animal Sciences* 25, 1255–1261.
- Rouse S, van Sinderen D (2008): Bioprotective potential of lactic acid bacteria in malting and brewing. *Journal of Food Protection* 71, 1724–1733.

- Signorini ML, Zbrun MV, Sequeira GJ, Rosmini MR, Frizzo LS (2012): Impact of probiotic administration on the health and fecal microbiota of young calves: A meta-analysis of randomized controlled trials of lactic acid bacteria. *Research in Veterinary Science* 93, 250–258.
- Silveira MS, Alexandre CPML, Guilherme A, Fernandes FAN, Rodrigues S (2012): Cashew apple juice as substrate for Lactic Acid production. *Food and Bioprocess Technology* 5, 947–953.
- Stanton AL (2014): Finding the future now – Health, genomics, and calves. In: ADSA-ASAS Midwest Meeting. American Society of Animal Science. 18–19.
- Tsuda H, Teruki M, Yoshiko I (2012): Selection of lactic acid bacteria as starter cultures for fermented meat products. *Food Science and Technology Research* 18, 713–721.
- Tsuruta T, Inoue R, Tsukahara T, Matsubara N, Hamasaki M, Ushida K (2009): A cell preparation of *Enterococcus faecalis* strain EC-12 stimulates the luminal immunoglobulin A secretion in juvenile calves. *Animal Science Journal* 80, 206–211.
- Zhao K, Qiao Q, Chu D, Gu H, Ha Dao T, Zhang J, Bao J (2013): Simultaneous saccharification and high titer lactic acid fermentation of corn stover using a newly isolated lactic acid bacterium *Pediococcus acidilactici* DQ2. *Bioprocess Technology* 135, 481–489.

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