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Applicability of *Pediococcus* strains for fermentation of cereal bran and its influence on the milk yield of dairy cattle

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Abstract

The isolated *Pediococcus acidilactici* BaltBio01 and *Pediococcus pentosaceus* BaltBio02 strains were cultivated in barley and wheat bran (90/10, m/m) substrate, and the developed fermented feed stock, with high content of valuable *Pediococcus*, was used for Lithuanian black and white dairy cattle feeding. In addition, the influence of fermented feed stock on milk production and composition was determined.

Isolated strains demonstrated versatile carbohydrate metabolism, grown at 30°C and 37°C temperatures, acidic tolerance, showed to be non resistant to antibiotics and have antimicrobial activity. Bioconversion of cereal by-products (barley and wheat bran) using isolated microorganisms allows production of safer (reduced *Enterobacteria*, aerobic bacteria, yeast and mould count) fermented feed stock with high content of *Pediococcus*. At the beginning of the feeding experiment, no significant differences in feed intake, milk production and milk composition were established between the test groups. Control and trial groups received the same ration; however, the trial groups, in addition, received 200 g per dairy cattle of the fermented feed stock containing 9.6 $\log_{10}$ CFU g^{-1} *Pediococcus* daily for 66 days (group B received 200 g fermented with *P. acidilactici*/dairy cattle/day feed stock, group C received 200 g fermented with *P. pentosaceus*/dairy cattle/day feed stock, and group D received 200 g fermented with *P. acidilactici* and *P. pentosaceus*/dairy cattle/day feed stock). After 33 days of experiment, significant milk yield increase in group D (yield 34.64 ± 3.56 kg d^{-1}), compared with group A (yield 29.93 ± 3.55 kg d^{-1}) and group C (yield 30.24 ± 3.99 kg d^{-1}) was established. After 66 days of feeding, similar trends were found, i.e. milk yield significantly increased in group D (yield 35.04 ± 3.04 kg d^{-1}) compared with group A (yield 29.04 ± 3.88 kg d^{-1}).

It can be inferred that barley and wheat bran could be used to promote growth of *P. acidilactici* BaltBio01 and *P. pentosaceus* BaltBio02, and the produced fermented feed stock could be recommended for dairy cattle feeding in order to increase milk production.

Key words: barley and wheat bran, dairy cattle, fermented feed stock, milk, *Pediococcus*.

Introduction

The environmental impact of agricultural by-products from the processing of food crops is an increasing concern worldwide. Currently, cereal bran has been used as a low-value ingredient for both human consumption and animal feed. The most popular bioprocessing technologies for cereal bran nutritional and technological functionality increasing are enzymatic processing and fermentation (Coda et al., 2015), and the most popular starters in fermented feed production are lactic acid bacteria (LAB) including *Pediococci*, which constitute the most suitable choice for application as protective cultures, since they are present in all fermented substrates with long history of safe use, and form part of the gut microflora of humans and animals (Allen et al., 2013). The scientific community has proposed alternatives to the use of antibiotics, including the use of microorganisms showing positive effect on animal health and productivity parameters (Redondo et al., 2014). In adult ruminants, probiotics have mostly been selected to target the rumen compartment, which is the main site of feed digestion (Chaucheyras-Durand, Durand, 2010). The use of probiotics proved to be effective in increasing milk production of lactating dairy cattle (Vibhute et al., 2011; Salvedia et al., 2015; Shreedhar et al., 2016). Gradual improvement was observed in overall milk composition with supplementation of probiotics (Vibhute et al., 2011).

However, the ruminant digestive system is unique, there are billions of microorganisms which help the dairy cattle to digest and utilize nutrients in the feed. To achieve efficient feed utilization and high milk yield, the microorganisms must have optimal conditions, and the disbalance of this system is highly undesirable. Bacterial antagonism is a common phenomenon in nature; therefore, microbial interactions play a major role in the equilibrium between competing beneficial and potentially pathogenic microorganisms (Balcazar et al., 2006). Most microorganism based products utilize one or more of several types of bacteria. Antimicrobial activities of LAB are due to organic acids, hydrogen peroxide and

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bacteriocins production (Dimitonova et al., 2008). The benefit of many bacteriocins is their low oral toxicity for the treated host. Indeed, many bacteriocins produced by LAB, in particular, have been consumed in fermented feeds as a feed preservative. The same principles apply to bacteriocin producing cultures and their application to control pathogens in the gut (Connor et al., 2015).

According to the FAO/WHO (2006), the development of commercial microbial feed supplements requires their unequivocal taxonomic identification, as well as their *in vitro* and *in vivo* functional characterization and safety assessment. In Europe, the European Food Safety Authority (EFSA) proposed a system for a pre-market safety assessment of selected groups of microorganisms used in feed and the production of feed additives leading to a Qualified Presumption of Safety (QPS) status (EFSA, 2011). According to the EFSA approach, most LAB species are included in the QPS list and, therefore, demonstration of their safety only requires confirmation of the absence of determinants of resistance to antibiotics of human and veterinary clinical significance (Muñoz-Atienza et al., 2013). Evidence of the harmlessness of the probiotic to the environment is one important subject for its registration. In general, any negative impact is highly unlikely since all these microorganisms are derived from nature (Yirga, 2015).

The aim of this study was to use spontaneously fermented rye, as a source of the lactic acid bacteria, for the isolation of new strains, and by using isolated strains to develop sustainable technology for fermented feed stock production. In addition, to investigate the influence of developed fermented feed stock on the milk production of Lithuanian black and white dairy cattle.

Materials and methods

The isolation, identification, and characterisation of the microorganisms were conducted in 2015 at the University of Natural Resources and Life Sciences (BOKU) in Vienna, Austria. Other analyses and experiments with animals were conducted in 2016 at the Lithuanian University of Health Sciences.

Production of spontaneously fermented rye. Spontaneously fermented rye was produced by using the following scheme: 100 g rye flour + 1% acetic acid + 1% salt + 150 ml water → fermentation 48 h at 30°C → + 50 g rye flour + 50 ml water → fermentation 24 h at 30°C.

Purification, isolation and identification of Pediococcus by polymerase chain reaction (PCR)-based analysis techniques. Purification of cells was performed according to Kiss et al. (2007). The molecular fingerprinting of the final strains was done by rep-typing with the primer GTG5 (5'-GTG GTG GTG GTG GTG-3') (Versalovic et al., 1994). Polymerase chain reaction (PCR) was carried out in a Master Cycler (Eppendorf, Germany). The resulting (GTG)5-PCR fingerprints were analyzed using the software package *BioNumerics v4.0* (Applied Maths, Belgium). 16S rDNA sequencing was conducted for selected strains by applying the primers – Bak4 (5'-AGG AGG TGA TCC ARC CGC A-3'), Bak11 (5'-AGT ATTG ATC MTG GCT CAG -3'), set and PCR protocol published by Di Cello et al. (1997). The PCR products were purified applying the peqGold-Cycle-Pure Kit (Peqlab Biotechnology GmbH, Germany) and sequenced (Eurofins MWG Operon, Austria). The received sequences were analysed with the BLASTn tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi), and a minimum sequence identity of 98% was chosen as a criterion for species identification. Colonies were identified as belonging to the genus *Pediococcus* (Pfannebecker, Fröhlich, 2008) by means of PCR. The PCR-based identification on species level followed the strategy by Mora et al. (1997).

Evaluation of isolated Pediococcus carbohydrate metabolism, gas production, tolerance to temperature and low pH conditions. Carbohydrate fermentation profiles of the strains were determined by using API 50 CH system (BioMerieux, France). Moreover, each pure culture was further characterized by Durham tube method in de Man, Rogosa and Sharpe (MRS) broth at 30°C for 24 h for detecting gas production. The growth performances at 30, 37 and 45 °C for 24 h in MRS broth were monitored using a Thermo Bioscreen C automatic turbidometer (Labsystems Oy, Finland). The ability of the strains to survive at low pH was evaluated in triplicate as described by Lee et al. (2011), in acidified MRS broth (final pH 2.5).

The evaluation of Pediococcus resistance to antibiotics. The minimum inhibitory concentrations (MICs) of ten antibiotics: gentamicin (GEN), kanamycin (KAN), streptomycin (STREP), tetracycline (TET), erythromycin (ERY), clindamycin (CLIN), chloramphenicol (CHL), ampicillin (AMP), amoxicillin (AML) and trimethoprim (TM), were determined by E-test method. Interpretation of the results was performed according to the guidance on the assessment of bacterial antimicrobial susceptibility described by EFSA (EFSA-FEEDAP, 2012).

The evaluation of antimicrobial activities of isolated Pediococcus. The *Pediococcus* (*P. acidilactici* BaltBio01 and *P. pentosaceus* BaltBio02) were grown in MRS medium (Biolife, Italy) at their optimal temperatures (*P. acidilactici* at 32°C, *P. pentosaceus* at 35°C). Two percent of *Pediococcus* cells were inoculated into a fresh medium and propagated for 18 h. The cells were harvested by centrifugation (6000 g, 10 min and 4°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 and its mix against variety of pathogenic and opportunistic bacterial strains (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enterica, Corynebacter* spp., *Klebsiella pneomoniae*, *Enterococcus faecalis* and *Bacillus cereus*) previously isolated from diseased cattle. Agar well diffusion assay was used for antimicrobial activities testing. For this purpose, 0.5 McFarland unit density suspension of each pathogenic bacterial strain was inoculated onto surface of cooled Mueller Hinton agar (Oxoid Ltd, 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 of inhibition zones was calculated. The antimicrobial activities against tested bacteria were determined by measuring the diameter (mm) of inhibition zones.

Preparation of barley and wheat bran fermented feed stock with high content of viable P. acidilactici BaltBio01 and P. pentosaceus BaltBio02. Mix of barley and wheat bran (moisture content 11.6%; 90/10, m/m) (Biglio dribsnių gamyba Ltd, Lithuania) was used as the fermentation medium for *P. acidilactici* and *P. pentosaceus*. Before the experiment, *Pediococcus* strains were stored at −80°C in a Microbank system (Pro-Lab Diagnostics, UK) and were propagated in MRS agar CM0359 (Oxoid Ltd, UK) at 30°C for 48 h with the

addition of 40 mM fructose and 20 mM maltose prior to use. The bacteria were diluted with a physiological saline to a concentration of 10^8 CFU mL^{-1} before the cereal bran fermentation.

The fermented product (45% moisture content) was prepared using 100 kg of cereal bran (barley and wheat bran, 90/10, m/m) and 37 L of water. LAB cell suspension (3%, m/m) containing about 10^8 CFU mL^{-1} was added, followed by fermentation for 72 h at temperature optimal for the strains (*P. acidilactici* at 32°C, *P. pentosaceus* at 35°C). Bran substrate with high content of *Pediococcus* (10^9 CFU g^{-1}) was divided into the 200 g portions and used for dairy cattle feeding. A new portion of fermented feed stock was prepared every 7 days, because *Pediococcus* count in high moisture bran substrate during the 7 days' period (storage temperature +4°C) was stable (10^9 CFU g^{-1}). Fermented feed stock containing *P. acidilactici* and *P. pentosaceus* strains was produced by mixing fermented with *P. acidilactici* feed stock and fermented with *P. pentosaceus* feed stock in equal parts (50/50, m/m).

Microbiological analysis of the fermented cereal bran. For microbiological analysis 10 g of sample were homogenized with 90 mL of saline (0.9%). The suspension was diluted, and the 10^{-4}–10^{-8} solutions were inoculated in MRS agar CM0361 (Oxoid Ltd, UK) and incubated under anaerobic conditions at 35°C for 72 h (for *Pediococcus* strains). MacConkey agar CM0007B (Oxoid Ltd, UK) was used for determination of total count of *Enterobacteria*, the Nutrient agar (Biolife Italiana Srl, Italy) was used for total aerobic bacteria, and the Sabouraud glucose agar C974Q82 (Sigma-Aldrich, Germany) was used for fungi, followed the incubation under aerobic conditions at 37°C for 72 h. A final number of bacteria was calculated and expressed as a $\log_{10}$ of colony forming units (CFU) per gram of sample. Three replications per treatment were prepared.

Experimental animals and diets, milk samples collection and analyses. Effects of *P. acidilactici* and *P. pentosaceus* fermented feed stock and its mix were investigated on 160 mid-lactation dairy cattle divided into four groups each containing 40 animals: control (A) and trial (B, C and D) animals were cared for according to the Requirements for keeping, maintenance and use of animals intended for experimental and other scientific purposes (Valstybės žinios, 2012). The experiment was performed in the winter at a farm of Lithuanian black and white dairy cattle. Division of the dairy cattle into groups was based on parity, body weight and stage of the lactation cycle. All dairy cattle passed general health examinations monthly throughout. Control and trial groups received identical diet (Table 1). Nutrient composition in 1 kg of diet in dry matter: net energy lactation 7.33 MJ, crude protein 17.0%, crude fat 5.70%, crude fibre 15.0%.

Table 1. Ingredients of the diets fed to dairy cattle

Diet composition	Weight kg	Diet composition	Weight kg
Haylage	14.0	Mineral supplement	0.35
Corn silage	12.0	Sugar beet pulp	2.5
Haulm	0.5	Corn grain silage	3.5
Molasses	1.0	Salt	0.05
Triticale	2.5	Corn grain	2.0
Soybean meal	1.5	Soda	0.15
Rapeseed expeller	2.0	Protected fat	0.3

However, the trial groups additionally received 200 g per dairy cattle of the fermented feed stock daily during 66 days (group B received 200 g fermented feed stock containing 9.6 $\log_{10}$ CFU g^{-1} of *P. acidilactici*/dairy cattle/day, group C received 200 g fermented feed stock containing 9.6 $\log_{10}$ CFU g^{-1} of *P. pentosaceus*/dairy cattle/day, group D received 200 g fermented feed stock containing 9.6 $\log_{10}$ CFU g^{-1} of *P. acidilactici*/dairy cattle/day and *P. pentosaceus*/dairy cattle/day). The fermented feed stock supplements were fed individually. Control group received 200 g nonfermented barley and wheat bran (90/10, m/m). The daily feed intake on a dry matter (DM) basis was determined by the measurement of differences between the feed offered and orts. Orts were collected every day. Water was available *ad libitum*.

Milk samples from all experimental dairy cattle (n = 160) were collected and daily milk yields were recorded during control milking three times: first – at the beginning of the experiment, i.e. one day before starting the feeding of the fermented feed stock supplement, second – after 33 days of the feeding of the fermented feed stock supplement, and third – after 66 days of the feeding of the fermented feed stock supplement. Milk yield adjusted to 4% fat-corrected milk (FCM) was calculated with the following equation: 4% FCM = 0.4 × actual milk yield (in kg d^{-1}) + 15 × milk fat (in kg d^{-1}). The following parameters of milk were measured: milk fat, milk protein, milk lactose, milk urea by the equipment LactoScope FTIR, FT1.0. 2001 (Delta Instruments, Holland).

Statistical analysis. The experimental data were evaluated statistically and presented as mean ± standard error of the mean (mean ± SEM). The significance of arithmetic differences (p) was defined according to the *Student's t*-test. In order to evaluate the influence of different factors (type of *Pediococcus* strain and duration of feeding with fermented feed stock supplements) and their interaction on dairy cattle milk production, the data were subjected to two-way analysis of variance (*ANOVA*), statistical program *R 3.2.1* (*R* Core Team, 2015). The results were referred to statistically significant at $p < 0.05$.

Results and discussion

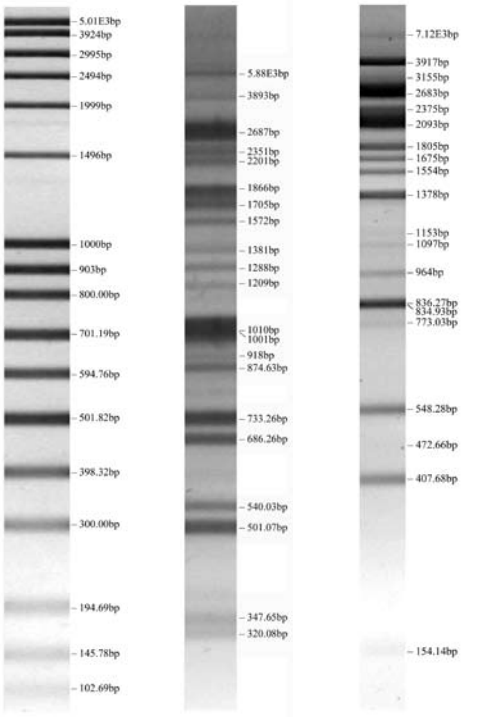
The properties of isolated Pediococcus strains. Identification of the isolated *Pediococcus* strains by using the software package *BioNumerics v4.0*, as well as carbohydrate metabolism, gas production, tolerance to temperature and low pH conditions (pH 2.5 for 2 h of incubation) are shown in Table 2.

Many factors contribute to a successful fermentation of carbohydrate-rich feeds, and metabolic activities of used microorganisms play a leading role. All the tested strains were able to ferment L-arabinose, D-ribose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, N-acetylglucosamine, amygdalin, arbutin, esculin, salicin, D-cellobiose, D-trehalose, gentiobiose and D-tagatose. Some differences between carbohydrate metabolism in the analyzed *Pediococcus* strains were observed, only *P. acidilactici* was able to ferment L-rhamnose, methyl-αD-mannopyranoside and potassium gluconate, and only *P. pentosaceus* showed high activity (+++) of D-maltose, D-melibiose, D-saccharose, D-raffinose and low activity (+) of potassium 2-ketogluconate metabolism. The use of *Pediococcus* strains showing versatile carbohydrate metabolism, can be of great advantage to the animal, since no digestive enzymes exist for breaking down cellulose or other complex carbohydrates.

All the tested strains were grown at 30°C and 37°C, as well as *P. acidilactici* were grown at 45°C, and after 2 h of incubation at 2.5 pH, the viable count of

Table 2. Bands of isolated *Pediococcus* strains (*P. acidilactici* BaltBio01 and *P. pentosaceus* BaltBio02) genus, their carbohydrate metabolism, gas production, tolerance to temperature and low pH

Parameters		*Pa*	*Pp*
L-arabinose		+++	+++
D-ribose		+++	+++
D-xylose		+++	+++
D-galactose		+++	+++
D-glucose		+++	+++
D-fructose		+++	+++
D-mannose		+++	+++
L-rhamnose		+++	−
Methyl-αD-mannopyranoside		++	−
N-acetylglucosamine		++	+++
Amigdalin		++	+++
Arbutin		++	+++
Esculin		+++	+++
Salicin		+++	+++
D-cellobiose		+++	+++
D-maltose		−	+++
D-melibiose		−	+++
D-saccharose		−	+++
D-trehalose		+++	+++
D-raffinose		−	+++
Gentiobiose		++	++
D-tagatose		+++	+++
Potassium gluconate		+	−
Potassium 2-ketogluconate		−	+
Gas production (+/−)		−	−
Tolerance to temperature	30°C	+	+
	37°C	+	+
	45°C	+	−
Acidified MRS broth (pH 2.5)	After 0 h $\log_{10}$ CFU mL^{-1}	8.5 ± 0.2	7.9 ± 0.2
	After 2 h $\log_{10}$ CFU mL^{-1}	4.3 ± 0.1	4.8 ± 0.1



Pa – *P. acidilactici*, *Pp* – *P. pentosaceus*; +++ – high growth (yellow), ++ – quite growth (green), + – little growth (dark green), − – not growth (blue)

P. acidilactici was found to be 4.3 ± 0.1 $\log_{10}$ CFU mL^{-1} and *P. pentosaceus* was 4.8 ± 0.1 $\log_{10}$ CFU mL^{-1} (Table 2). Gas production of tested strains was not observed.

P. acidilactici BaltBio01 and P. pentosaceus BaltBio02 resistance to antibiotics. Modification of rumen fermentation by using feed additives, such as antibiotics, has proved to be a useful strategy to improve production efficiency in ruminants. However, the use of antibiotics has been increasing concern due to the potential appearance of residues in milk, meat and bacterial resistance. Furthermore, the use of antibiotics as a feed additive has been banned in the European Union (Russell, Houlihan, 2003). According to EFSA, as a basic requirement, the minimum inhibitory concentration of the antimicrobials should be determined for each of the following substances: GEN, KAN, STREP, TET, ERY, CLIN, CHL and AMP, also, resistance to AML and TM is very important (EFSA-FEEDAP, 2012). When a bacterial strain demonstrates higher resistance to a specific antimicrobial than the other strains of the same taxonomical unit, the presence of acquired resistance is indicated and additional information is needed on the genetic basis of the antimicrobial resistance. Any bacterial strain carrying an acquired resistance to antimicrobial that is shown to be due to the acquisition of genetic determinant presents the greatest potential for horizontal spread and should not be used as a feed additive. *P. acidilactici* BaltBio01 and *P. pentosaceus* BaltBio02 resistance to antibiotics is shown in Table 3.

The *Pediococcus* strains were considered non resistant to all EFSA recommended antibiotics, when the MIC (mg mL^{-1}) values obtained were lower than the recommended breakpoint value, defined at species level by the FEEDAP (EFSA-FEEDAP, 2012). In the present study, *P. acidilactici* and *P. pentosaceus* were non resistant to GEN, KAN, STREP, TET, ERY, CLIN, CHL and AMP. Also, *P. acidilactici* and *P. pentosaceus* were non resistant to AML and TM, MIC values of *P. acidilactici* were 0.25 and 32 mg mL^{-1}, and of *P. pentosaceus* – 0.75 and 32 mg mL^{-1}, respectively. *Pediococcus* strains used in the experiment showed to be non resistant to the tested antibiotics, and from this point of view these strains are safe for the use as starter cultures for fermented feed stock production.

Antibiotics are a major tool utilized by the health care industry to fight bacterial infections; however, bacteria are highly adaptable creatures and are capable of developing resistance to antibiotics. Consequently, decades of antibiotic use, or rather misuse, have resulted in bacterial resistance to many modern antibiotics. This antibiotic resistance can cause significant danger and suffering for many people and animals with common bacterial infections, those once easily treated with antibiotics. For several decades, studies on selection and dissemination of antibiotic resistance have focused mainly on clinically relevant species. However, recently many researchers have investigated commensal bacteria including LAB, which may act as reservoirs of antibiotic resistance genes similar to those found in human pathogens. The main threat associated with these bacteria is that they can transfer resistance genes to pathogenic bacteria.

Antimicrobial activities of P. acidilactici and P. pentosaceus and their mix. One of the most important modes of action of LAB is antimicrobial activity through inhibition of the pathogenic bacteria (Yang et al., 2015). Antimicrobial activities of *P. acidilactici* and

Table 3. Inhibition of the growth of pathogenic bacteria by *Pediococcus acidilactici*, *P. pentosaceus* and mix of *P. acidilactici* with *P. pentosaceus*, and their resistance to antibiotics

Microorganisms	Zone of inhibition mm			*Anti-biotics*	Resistance to antibiotics		
					Pa	*Pp*	FEEDAP
	Pa	*Pp*	*Pa* × *Pp*		MIC mg mL^{-1}		breakpoint mg mL^{-1}
				GEN	12	14	16
				TET	2.5	2	8
Pseudomonas aeruginosa	15.9 ± 0.5	17.3 ± 0.6	18.4 ± 0.3	ERY	0.19	0.25	1
Staphylococcus aureus	16.3 ± 0.6	16.4 ± 0.5	17.2 ± 0.5	AML	0.25	0.75	nr
Escherichia coli	15.8 ± 0.3	13.7 ± 0.3	16.1 ± 0.4	TM	32	32	nr
Salmonella enterica	9.6 ± 0.4	11.3 ± 0.3	13.8 ± 0.4	KAN	32	24	64
Corynebacterium spp.	11.4 ± 0.3	12.7 ± 0.4	13.9 ± 0.3	STREP	48	48	64
Klebsiella pneumoniae	11.3 ± 0.3	11.6 ± 0.6	12.0 ± 0.6	CLIN	0.75	0.25	1
Enterococcus faecalis	14.7 ± 0.5	13.3 ± 0.3	15.2 ± 0.4	CHL	3	2	4
Bacillus cereus	11.4 ± 0.3	14.5 ± 02	15.4 ± 0.5	AMP	1.5	1	4

Notes. Values are mean of three replicate analyses. *Pa* – *P. acidilactici*, *Pp* – *P. pentosaceus*; *Pa* × *Pp* – mix of *P. acidilactici* with *P. pentosaceus*; GEN – gentamicin, TET – tetracycline, ERY – erythromycin, AML – amoxicillin, TM – trimethoprim; KAN – kanamycin, STREP – streptomycin, CLIN – clindamycin, CHL – chloramphenicol, AMP – ampicillin; MIC – minimum inhibitory concentration; FEEDAP – Panel on Additives and Products or Substances used in Animal Feed Breakpoint (EFSA-FEEDAP, 2012); nr – not required.

P. pentosaceus and their mix are presented in Table 3. As could be seen from the results obtained, LAB supernatants inhibited the growth of all tested bacteria. The diameters of the inhibition zones of *P. acidilactici* toward pathogenic strains varied between 9.6 ± 0.4 and 16.3 ± 0.6 mm, of *P. pentosaceus* – between 11.3 ± 0.3 and 17.3 ± 0.6 mm, and mix of *P. acidilactici* with *P. pentosaceus* – between 12.0 ± 0.6 and 18.4 ± 0.3 mm. In all the cases, higher antimicrobial activity demonstrated mix of *P. acidilactici* with *P. pentosaceus*, in compare with separate *Pediococcus* strains. The highest inhibition zones of *P. acidilactici* and *P. pentosaceus* mix were observed against *Pseudomonas aeruginosa* (inhibition zone diameter was 18.4 ± 0.3 mm); the lowest inhibition zones were observed against *Klebsiella pneumoniae* (12.0 ± 0.6 mm).

Lactic acid bacteria (LAB) are characterised by many properties to control pathogens. These properties contain competence with pathogens in cell attachment, high adherence ability to intestinal epithelium, production of antibacterial substances such as organic acids, hydrogen peroxides, bacteriocins, etc. (Kermanshahi et al., 2015). Bacteriocins contribute to the biological control of pathogenic microorganisms. These metabolic compounds probably act synergistically (Choffnes et al., 2012). Bacteriocin production can be one of the most important factors for the successful establishment of probiotic bacteria (Asurmendi et al., 2015; Kermanshahi et al., 2015). Our previous studies showed, that *Pediococcus* strains, used in this experiment for dairy cattle feeding, produced bacteriocins (*P. acidilactici* produced pediocin Ac05-7 and *P. pentosaceus* produced pediocin 05-8), and inhibited most of the food pathogenic bacteria (Cizeikiene et al., 2013). Also, data are published that the *P. pentosaceus* bacteriocin was active over a wide range of pH values and was stable to various heat treatments. This heat and pH stability may be useful if the bacteriocin is to be used as an antimicrobial agent in fermented or thermally processed substrates (Osmanagaoglu et al., 2011). This study showed that the higher antimicrobial activity could be achieved by using mix of selected *Pediococcus* strains, compared to separate strains usage.

Microbiological parameters and pH of fermented feed stock. The microbiological parameters and pH of the fermented feed stock are presented in Table 4. The count of LAB in the samples after 72 h of fermentation with *P. acidilactici* was 8.9 ± 0.1 log10 CFU g^{-1}, and after 72 h of fermentation with *P. pentosaceus* was 9.6 ± 0.4 log10 CFU g^{-1}. Fermentation inhibited growth of *Enterobacteria* and reduced the growth of total aerobic bacteria, yeast and mould in barley and wheat bran substrate. Reduction of mould growth in the fermented cereal bran samples was lower compared to yeast, but statistically significant ($p \leq 0.05$).

The homo-fermentative LAB produce lactic acid as the major metabolic end product of carbohydrate fermentation, which leads to substrate pH reduction, and these changes have influence on growth inhibition of many microorganisms (Kermanshahi et al., 2015; Yang et al., 2015).

LAB are characterized by their tolerance to low pH and growth under this circumstance in which other bacteria are unable to grow, and thereby ensuring safety of fermented feed. Furthermore, penetration of lactic acid to the membranes leads to lowering the intracellular pH (Kermanshahi et al., 2015). The lowest pH was found after 72 h of fermentation. The results of the *ANOVA* test indicated that there was a significant effect of duration of fermentation on LAB count, total aerobic bacteria count, *Enterobacteria* count and yeast count ($p = 0.0001$) in barley and wheat substrate. However, significant influence of duration of fermentation on mould count was not found.

The hetero-fermentative LAB produces lactic acid and additional products such as acetic acid, ethanol and carbon dioxide. Ethanol is a part of intermediary products that are converted into produce CO_2 and H_2. Carbon dioxide interacts with cell membranes by reducing internal and external pH levels. Diacetyl is a product of citrate metabolism, and peroxide oxidizes membrane lipids and cell proteins (Choffnes et al., 2012; Kermanshahi et al., 2015). Also, bacteriocins produced by LAB affect membranes, DNA synthesis and protein synthesis. *P. pentosaceus* and *P. acidilactici* strains have capability to produce bacteriocins (Cizeikiene et al., 2013). Bacteriocins contribute to the biological control of pathogenic and spoilage microorganisms. The results of the *ANOVA* test indicated that there was a significant effect of the type of *Pediococcus* used for the cereal bran fermentation on substrate pH ($p = 0.0001$), LAB count ($p = 0.0001$), total aerobic bacteria count ($p = 0.0001$), *Enterobacteria* count ($p = 0.0001$), yeast count ($p = 0.0002$) and mould count ($p = 0.0004$). The interaction between

Table 4. Microbiological parameters of fermented feed stock

Samples	Fermentation time h	Lactic acid bacteria	Total aerobic bacteria	*Enterobacteria*	Fungi	
					yeast	mould
		$\log_{10}$ CFU g^{-1}				
Nonfer-mented	0	4.6 ± 0.1 a	5.4 ± 0.1 c	5.9 ± 0.2	6.1 ± 0.2 d	4.6 ± 0.1 b
Pa	24	8.2 ± 0.3 d	4.3 ± 0.2 b	–	5.5 ± 0.3 c	4.2 ± 0.4 a
	48	9.4 ± 0.1 d	4.3 ± 0.3 b	–	5.3 ± 0.1 c	4.0 ± 0.2 a
	72	8.9 ± 0.1 d	4.7 ± 0.1 b	–	5.1 ± 0.3 a	4.0 ± 0.1 a
Pp	24	8.4 ± 0.3 b	4.4 ± 0.2 b	–	5.7 ± 0.3 c	4.4 ± 0.2 a
	48	9.3 ± 0.2 d	4.6 ± 0.4 b	–	5.4 ± 0.4 b	4.3 ± 0.3 a
	72	9.6 ± 0.4 d	4.6 ± 0.3 b	–	5.2 ± 0.2 a	4.3 ± 0.2 a
Pa × *Pp*	24	7.7 ± 0.2 b	4.4 ± 0.3 b	–	5.8 ± 0.2 c	4.0 ± 0.3 a
	48	8.6 ± 0.2 d	4.6 ± 0.2 b	–	5.2 ± 0.1 b	3.9 ± 0.1 a
	72	9.8 ± 0.3 d	4.4 ± 0.1 b	–	5.1 ± 0.3 a	3.9 ± 0.1 a

Notes. Pa – fermented with *P. acidilactici*, *Pp* – fermented with *P. pentosaceus*, *Pa* × *Pp* – mix of *P. acidilactici* with *pentosaceus*; CFU – colony forming units. Values are mean of three replicate analyses. Different letters indicate significant differences between mean values of treatments ($p < 0.05$).

the analysed factors (duration of fermentation and type of *Pediococcus* used for cereal bran fermentation) was determined as statistically significant on the LAB count ($p < 0.0019$) and pH ($p < 0.0001$) of cereal bran substrate.

The influence of P. acidilactici, P. pentosaceus and mix of P. acidilactici with P. pentosaceus fermented feed stock supplements on dairy cattle feed intake, milk production and milk composition. The influence of (with *P. acidilactici*, *P. pentosaceus* and mix of *P. acidilactici* with *P. pentosaceus*) fermented feed stock supplements on dairy cattle feed intake, milk production and milk composition is shown in Tables 5 and 6.

At the beginning of the experiment (feeding day 0) no significant differences in feed intake, milk production and milk composition between the different groups of dairy cattle were established. After 33 days of feeding with fermented feed stock supplements statistically significant milk yield increase in group D (compared with group A and group C) was established ($p = 0.008$ and $p = 0.02$, respectively). After 66 days of feeding with fermented feed stock supplements similar trends were found, i.e. milk yield significantly ($p = 0.001$) increased in group D (compared to group A), and FCM significantly increased in groups B and C, compared to control group (A) ($p = 0.02$ and $p = 0.03$, respectively).

However, significantly higher FCM was established in group D, compared to groups B and C ($p = 0.05$ and $p = 0.03$, respectively). Dairy cattle production is generally expressed in terms of daily milk production, FCM or feed efficiency. The inclusion of microbial supplements in feed benefits the dairy cattle with already developed rumen by increasing palatability, stimulating cellulolytic bacteria and thus the rumen fermentation, improving nutrient digestibility in the rumen, preventing a decline in rumen pH by decreasing lactic acid production and/or increasing utilization of lactic acid by some bacteria and hence improve productivity (El-Din, Nour, 2015). Increased production of milk protein and fat might indicate that changes in rumen fermentation as a result of feeding with microorganisms enriched supplements increased the supply of glucogenic, aminogenic and lipogenic substrates (Erasmus et al., 2005). Explanation for increased synthesis of milk protein is a possible change in the profile of intestinally absorbed amino acids (Schwab et al., 1992). Our previous studies showed that by using *P. acidilactici* and *P. pentosaceus* strains it is possible to increase digestibility of soybean and to reduce activity of trypsin inhibitors (Bartkiene et al., 2015), therefore soybean meal was included in the diets of the investigated dairy cattle.

Table 5. Feed intake, milk production, and milk composition of dairy cattle fed with *Pediococcus acidilactici*, *P. pentosaceus* and mix of *P. acidilactici* with *P. pentosaceus* fermented feed stock supplements

Groups	Group A	Group B	Group C	Group D
Daily feed intake				
Total kg DM	20.01 ± 0.72	20.20 ± 0.70	20.14 ± 0.71	20.26 ± 0.58
Milk parameters				
Feeding day 0				
Yield kg d^{-1}	30.75 ± 5.18	29.50 ± 3.31	29.82 ± 4.59	30.03 ± 5.36
4% FCM kg d^{-1}	30.87 ± 4.16	30.01 ± 4.41	30.03 ± 4.28	30.39 ± 4.66
Fat %	4.08 ± 0.48	4.12 ± 0.64	4.08 ± 0.56	4.13 ± 0.52
Protein %	3.24 ± 0.25	3.22 ± 0.38	3.28 ± 0.39	3.27 ± 0.24
Lactose %	4.47 ± 0.15	4.48 ± 0.17	4.45 ± 0.13	4.53 ± 0.12
Urea mg dL^{-1}	15.20 ± 2.20	15.80 ± 2.74	15.50 ± 2.12	15.40 ± 2.32
Feeding day 33				
Yield kg d^{-1}	29.93 ± 3.55	31.42 ± 4.37	30.24 ± 3.99	34.64 ± 3.56
4% FCM kg d^{-1}	29.82 ± 2.49	32.03 ± 3.26	30.90 ± 3.75	35.38 ± 2.31
Fat %	4.01 ± 0.47	4.17 ± 0.47	4.19 ± 0.79	4.18 ± 0.50
Protein %	3.27 ± 0.16	3.38 ± 0.45	3.29 ± 0.57	3.28 ± 0.24
Lactose %	4.51 ± 0.13	4.43 ± 0.14	4.54 ± 0.12	4.53 ±0.10
Urea mg dL^{-1}	17.10 ± 2.92	16.80 ± 2.90	16.40 ± 1.84	16.30 ± 2.26
Feeding day 66				
Yield kg d^{-1}	29.04 ± 3.88	31.96 ± 4.27	31.74 ± 4.37	35.04 ± 3.04
4% FCM kg d^{-1}	29.16 ± 2.57	32.72 ± 3.70	32.52 ± 3.61	35.99 ± 3.14
Fat %	4.10 ± 0.82	4.20 ± 0.51	4.21 ± 0.49	4.21 ± 0.62
Protein %	3.40 ± 0.31	3.39 ± 0.48	3.35 ± 0.41	3.42 ± 0.33
Lactose %	4.55 ± 0.13	4.45 ± 0.22	4.48 ± 0.13	4.54 ± 0.13
Urea mg dL^{-1}	16.70 ± 2.36	16.20 ± 2.70	15.70 ± 2.11	17.00 ± 2.36

DM – dry matter, FCM – 4% fat-corrected milk; group A – control (basal diet), group B – basal diet plus supplement with *P. acidilactici*, group C – basal diet plus supplement with *P. pentosaceus*, group D – basal diet plus supplement with *P. acidilactici* and *P. pentosaceus* mix

Table 6. The influence of *Pediococcus acidilactici*, *P. pentosaceus* and mix of *P. acidilactici* with *P. pentosaceus* supplements on feed intake, milk production and milk composition of dairy cattle

	p-value								
Differences between groups	A *vs* B	A *vs* C	A *vs* D	B *vs* C	B *vs* D	C *vs* D	B d0 *vs* d33 / B d0 *vs* d66	C d0 *vs* d33 / C d0 *vs* d66	D d0 *vs* d33 / D d0 *vs* d66
Daily feed intake									
Total kg DM	0.35	0.52	0.19	0.78	0.76	0.56	–	–	–
Milk parameters									
Feeding day 0									
Yield kg d^{-1}	0.53	0.68	0.77	0.86	0.79	0.93	–	–	–
4% FCM kg d^{-1}	0.66	0.66	0.81	0.99	0.86	0.86	–	–	–
Fat %	0.88	0.99	0.84	0.90	0.97	0.86	–	–	–
Protein %	0.90	0.76	0.79	0.72	0.74	0.92	–	–	–
Lactose %	0.87	0.71	0.32	0.61	0.46	0.15	–	–	–
Urea mg dL^{-1}	0.60	0.76	0.85	0.79	0.73	0.92	–	–	–
Feeding day 33									
Yield kg d^{-1}	0.41	0.86	0.008	0.54	0.09	0.02	0.28	0.83	0.04
4% FCM kg d^{-1}	0.11	0.46	0.001	0.48	0.02	0.005	0.26	0.63	0.007
Fat %	0.45	0.54	0.45	0.55	0.98	0.96	0.83	0.72	0.83
Protein %	0.48	0.92	0.92	0.71	0.55	0.96	0.39	0.96	0.88
Lactose %	0.20	0.65	0.74	0.10	0.10	0.87	0.46	0.14	0.91
Urea mg dL^{-1}	0.82	0.53	0.50	0.72	0.67	0.91	0.44	0.32	0.39
Feeding day 66									
Yield kg d^{-1}	0.13	0.16	0.001	0.91	0.08	0.07	0.17	0.35	0.02
4% FCM kg d^{-1}	0.02	0.03	0.001	0.90	0.05	0.03	0.15	0.18	0.006
Fat %	0.76	0.73	0.75	0.96	0.97	1.00	0.77	0.61	0.77
Protein %	0.94	0.74	0.88	0.84	0.85	0.66	0.38	0.70	0.23
Lactose %	0.24	0.21	0.79	0.76	0.31	0.31	0.75	0.59	0.93
Urea mg dL^{-1}	0.66	0.33	0.78	0.65	0.49	0.21	0.75	0.84	0.14

DM – dry matter, FCM – 4% fat-corrected milk; group A – control (basal diet), group B – basal diet plus supplement with *P. acidilactici*, group C – basal diet plus supplement with *P. pentosaceus*, group D – basal diet plus supplement with *P. acidilactici* and *P. pentosaceus* mix; *p*-value contrast: A *vs* B = group A *vs* group B, A *vs* C = group A vs group C, A *vs* D = group A *vs* group D, B *vs* C = group B *vs* group C, B *vs* D – group B *vs* group D, C *vs* D – group C *vs* group D; B d0 *vs* d33 / B d0 *vs* d66 – day 0 *vs* day 33 and day 0 *vs* day 66 in group B; C d0 *vs* d33 / C d0 *vs* d66 – day 0 *vs* day 33 and day 0 *vs* day 66 in group C; D d0 *vs* d33 / D d0 *vs* d66 – day 0 *vs* day 33 and day 0 *vs* day 66 in group D

Conclusions

1. The isolated *Pediococcus acidilactici* BaltBio01 and *P. pentosaceus* BaltBio02 strains demonstrated versatile carbohydrate metabolism, grown at 30°C and 37°C, and acidic tolerance, also showed to be non resistant to the tested antibiotics and have antimicrobial activity against undesirable microorganisms.

2. Mixture of barley and wheat bran is a suitable substrate to produce safer (reduced *Enterobacteria*, total aerobic bacteria, yeast and mould count) feed with high content of *Pediococcus*.

3. Significantly higher milk yield (after 33 days) could be obtained by using *Pediococcus* mix (*P. acidilactici* BaltBio01 × *P. pentosaceus* BaltBio02) cultivated in cereal by-products for dairy cattle feeding.

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References

Allen H. K., Levine U. Y., Looft T., Bandrick M., CaseyT.A. 2013. Treatment, promotion, commotion: antibiotic alternatives in food-producing animals. Trends in Microbiology, 21 (3): 114–119 https://doi.org/10.1016/j.tim.2012.11.001

Asurmendi P., García M. J., Pascual L., Barberis L. 2015. Biocontrol of Listeria monocytogenes by lactic acid bacteria isolated from brewer's grains used as feedstuff in Argentina. Journal of Stored Products Research, 61: 27–31 https://doi.org/10.1016/j.jspr.2015.02.001

Balcazar J. L., de Blas I., Ruiz-Zarzuela I., Cunningham D., Vendrell D., Muzquiz J. L. 2006. The role of probiotics in aquaculture. Veterinary Microbiology, 114 (3): 173–186 https://doi.org/10.1016/j.vetmic.2006.01.009

Bartkiene E., Krungleviciute V., Juodeikiene G., Vidmantiene D., Maknickiene Z. 2015. Solid state fermentation with lactic acid bacteria to improve the nutritional quality of lupin and soya bean. Journal of the Science of Food and Agriculture, 95 (6): 1336–1342 https://doi.org/10.1002/jsfa.6827

Chaucheyras-Durand F., Durand H. 2010. Probiotics in animal nutrition and health. Beneficial Microbes, 1: 3–9 https://doi.org/10.3920/BM2008.1002

Choffnes E. R., Relman D. A., Olsen L., Hutton R., Mack A. 2012. Improving food safety through a one health approach: workshop summary. USA, 418 p.

Cizeikiene D., Juodeikiene G., Paskevicius A., Bartkiene E. 2013. Antimicrobial activity of lactic acid bacteria against pathogenic and spoilage microorganism isolated from food and their control in wheat bread. Food Control, 31 (2): 539–545 https://doi.org/10.1016/j.foodcont.2012.12.004

Coda R., Katina K., Rizzello C. G. 2015. Bran bioprocessing for enhanced functional properties. Current Opinion in Journal of Food Science, 1: 50–55 https://doi.org/10.1016/j.cofs.2014.11.007

Connor P. M. O., Ross R. P., Hill C., Cotter P. D. 2015. Antimicrobial antagonists against food pathogens: a bacteriocin perspective. Food Microbiology, 2: 51–57

Di Cello F., Bevivino A., Chiarini L., Fani R., Paffetti D., Tabacchioni S., Dalmastri C. 1997. Biodiversity of a *Burkholderia cepacia* population isolated from the maize rhizosphere at different plant growth stages. Applied and Environmental Microbiology, 63 (11): 4485–4493

Dimitonova P. S., Bakalov-Vladimirov B., Aleksandrova-Georgieva R. N., Trifonova D. S. 2008. Phenotypic and molecular identification of lactobacilli isolated from vaginal secretions. Journal of Microbiology, 41 (6): 469–477

EFSA. 2011. Maintenance of the list of QPS biological agents intentionally added to food and feed (2011 update). The EFSA Journal, 9 (12): 1–82

EFSA-FEEDAP. 2012. Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. EFSA Journal, 10 (6): 2740–2749 https://doi.org/10.2903/j.efsa.2012.2740

El-Din A. N. M., Nour M. I. L. K. 2015. Production and some blood metabolite responses to yeast supplementation in early lactating Holstein dairy cows. Egyptian Journal of Animal Production, 52 (1): 11–17

Erasmus L. J., Robinson P. H., Ahmadib A., Hinders R., Garrett J. E. 2005. Influence of prepartum and postpartum supplementation of a yeast culture and monensin, or both, on ruminal fermentation and performance of multiparous dairy cows. Animal Feed Science and Technology, 122: 219–239 https://doi.org/10.1016/j.anifeedsci.2005.03.004

FAO/WHO. 2006. Probiotics in food. Health and nutritional properties and guidelines for evaluation. FAO Food Nutrition Paper, 85: 1–50

Kermanshahi K. R., Hojati V., Shiravi A. 2015. Zinc oxide nanoparticles absorption rate in the heart tissue of female mice. Journal of Chemical Health Risks, 5 (3): 193–198
Kiss H., Kögler B., Petricevic L., Sauerzapf I., Klayraung S., Domig K. J., Viernstein, H., Kneifel W. 2007. Vaginal *Lactobacillus* microbiota of healthy women in the late first trimester of pregnancy. International Journal of Obstetrics and Gynaecology, 114 (11): 1402–1407 https://doi.org/10.1111/j.1471-0528.2007.01412.x
Lee J., Yun H. S., Cho K. W., Oh S., Kim S. H., Chun T. 2011. Evaluation of probiotic characteristics of newly isolated *Lactobacillus* spp.: immune modulation and longevity. International Journal of Food Microbiology, 148 (2): 80–86 https://doi.org/10.1016/j.ijfoodmicro.2011.05.003
Mora D., Fortina M. G., Parini C., Manachini P. L. 1997. Identification of *Pediococcus acidilactici* and *Pediococcus pentosaceus* based on 16S rRNA and ldhD gene-targeted multiplex PCR analysis. FEMS Microbiology Letter, 151 (2): 231–236 https://doi.org/10.1111/j.1574-6968.1997.tb12575.x
Muñoz-Atienza E., Gómez-Sala B., Araújo C., Campanero C., Campo del R., Hernández P. E., Herranz C., Cintas L. M. 2013. Antimicrobial activity, antibiotic susceptibility and virulence factors of Lactic Acid Bacteria of aquatic origin intended for use as probiotics in aquaculture. BMC Microbiology, 13: 15 https://doi.org/10.1186/1471-2180-13-15
Osmanagaoglu O., Kiran F., Nes I. F. 2011. A probiotic bacterium, *Pediococcus pentosaceus* OZF, isolated from human breast milk produces pediocin AcH/P A-1. African Journal of Biotechnology, 10 (11): 2070–2079
Pfannebecker J., Fröhlich J. 2008. Use of a species-specific multiplex PCR for the identification of *pediococci*. International Journal of Food Microbiology, 10 (2): 288–296 https://doi.org/10.1016/j.ijfoodmicro.2008.08.019
Redondo L. M., Chacana P. A., Dominguez J. E., Miyakawa M. E. F. 2014. Perspectives in the use of tannins as alternative to antimicrobial growth promoter factors in poultry. Frontiers in Microbiology, 5: 118 https://doi.org/10.3389/fmicb.2014.00118
Russell J. B., Houlihan A. J. 2003. Ionophore resistance of ruminal bacteria and its potential impact on human health. FEMS Microbiology Reviews, 27 (1): 65–74 https://doi.org/10.1016/S0168-6445(03)00019-6
Salvedia C. B., Supangco E. P., Vega R. S. A., Elegado F. B., Rayos A. A. 2015. Effect of probiotic feeding on milk yield and components of crossbred dairy goats. Philippine Journal of Veterinary and Animal Sciences, 41 (1): 21–30
Schwab C. G., Bozak C. K., Whitehouse N. L., Mesbah M. M. A. 1992. Amino acid limitation and flow to the duodenum at four stages of lactation. I. Sequence of lysine and methionine limitation. Journal of Dairy Sciences, 75 (12): 3486–3502 https://doi.org/10.3168/jds.S0022-0302(92)78125-9
Shreedhar J. N., Patil M., Gulbarga K. V. K., Kumar P. 2016. Effect of probiotics supplementation on milk yield and its composition in lactating Holstein Fresien and Deoni cross bred cows. Journal of Medical and Bioengineering, 5 (1): 19–23 https://doi.org/10.12720/jomb.5.1.19-23
Valstybės žinios, 2012-11-10, Nr. 130-6595 https://e-seimas.lrs.lt/portal/legalAct/lt/TAD/TAIS.437081 (in Lithuanian)
Versalovic J., Schneider M., De Bruijn F., Lupski J. R. 1994. Genome fingerprinting of bacteria using repetitive sequence-based polymerase chain reaction. Methods in Molecular and Cellular Biology, 5: 25–40
Vibhute V. M., Shelke R. R., Chavan S. D., Nage S. P. 2011. Effect of probiotics supplementation on the performance of lactating crossbred cows. Veterinary World, 4 (12): 557–561 https://doi.org/10.5455/vetworld.2011.557-561
Yang F., Hou C., Zeng X., Qiao S. 2015. The use of lactic acid bacteria as a probiotic in swine diets. Pathogens, 4 (1): 34–45 https://doi.org/10.3390/pathogens4010034
Yirga J. 2015. The use of probiotics in animal nutrition. Journal of Probiotics and Health, 3: 132 https://doi.org/10.4172/2329-8901.1000132

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Pediokokų kamienų panaudojimas grūdų sėlenų fermentacijai ir įtaka pieninių galvijų primilžiui

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Santrauka

Iš savaiminiu būdu fermentuotų rugių išskirtos *Pediococcus acidilactici* BaltBio01 ir *Pediococcus pentosaceus* BaltBio02 padermės buvo panaudotos miežinių ir kvietinių sėlenų (santykiu 90:10 %) fermentacijai. Pagaminta fermentuota pašarų žaliava, turinti didelį kiekį gyvybingų pediokokų, panaudota pieniniams galvijams (Lietuvos juodmargėms) šerti, ir įvertinta fermentuotos pašarų žaliavos įtaka pieninių galvijų primilžiui bei pieno sudėčiai. Pediokokai *P. acidilactici* BaltBio01 ir *P. pentosaceus* BaltBio02 fermentavo daugelį tirtų angliavandenių, gerai augo 30 ir 37 °C temperatūroje ir pasižymėjo atsparumu rūgštinei terpei. Išskirti pediokokai nebuvo atsparūs antibiotikams ir pasižymėjo antimikrobinėmis savybėmis. Grūdų gamybos šalutinių produktų (miežinių ir kvietinių sėlenų) biokonversija, taikant fermentaciją išskirtais mikroorganizmais, įgalina pagaminti saugesnę (su mažesniu *Enterobacteria*, aerobinių bakterijų, mielių ir pelėsių kiekiu) fermentuotą pašarų žaliavą, turinčią didelį kiekį gyvybingų pediokokų. Eksperimento pradžioje esminių skirtumų tarp pašaro suvartojimo, primilžio ir pieno sudėties nebuvo nustatyta. Eksperimento metu kontrolinės (A grupės) ir bandomųjų grupių racionas buvo vienodas, tačiau bandomosios grupės 66 dienas papildomai gavo po 200 g gyvuliui fermentuotos pašarų žaliavos, kurioje buvo 9,6 $\log_{10}$ KSV g^{-1} pediokokų (B grupės gyvuliai gavo po 200 g fermentuotos *P. acidilactici* pašarų žaliavos pieniniam galvijui/dieną, C grupė gavo po 200 g *P. pentosaceus* fermentuotos pašarų žaliavos pieniniam galvijui/dieną, o D grupė gavo po 200 g *P. acidilactici* ir *P. pentosaceus* fermentuotos pašarų žaliavos pieniniam galvijui/dieną). Po 33 dienų šėrimo nustatytas reikšmingas D grupės pieninių galvijų primilžio padidėjimas (34,64 ± 3,56 kg d^{-1}), lyginant su A (29,93 ± 3,55 kg d^{-1}) ir C (30,24 ± 3,99 kg d^{-1}) grupių pieninių galvijų primilžiu. Po 66 dienų šėrimo nustatytos panašios tendencijos, t. y. reikšmingai (p = 0.001) didesnis primilžis buvo D grupės pieninių galvijų (35,04 ± 3,04 kg d^{-1}), lyginant su A grupės pieninių galvijų primilžiu (29,04 ± 3,88 kg d^{-1}).
Apibendrinant galima teigti, kad miežinės ir kvietinės sėlenos gali būti naudojamos *P. acidilactici* BaltBio01 ir *P. pentosaceus* BaltBio02 padermėms gausinti, o didelį kiekį pediokokų turinti fermentuota pašarų žaliava gali būti rekomenduojama siekiant padidinti pieninių galvijų primilžį.

Reikšminiai žodžiai: fermentuota pašarų žaliava, miežinės ir kvietinės sėlenos, pediokokai, pienas, pieniniai galvijai.