Biblid: 1821-4487 (2020) 24; 2; p 57-61
UDK: 636.03
DOI: 10.5937/jpea24-26305

Original Scientific Paper
Originalni naučni rad

EFFECT OF LACTIC ACID FERMENTATION ON THE QUALITY OF BREWER'S SPENT GRAIN AS RUMINANT FEED

UTICAJ MLEČNO-KISELINSKE FERMENTACIJE NA KVALITET PIVSKOG TROPA KAO HRANIVA ZA PREŽIVARE

Dragana MLADENOVIĆ, Aleksandra ĐUKIĆ-VUKOVIĆ**, Jelena PEJIN***, Sunčica KOCIĆ-TANACKOV***, Milica RADOSAVLJEVIĆ****, Marija MILAŠINOVIĆ-ŠEREMEŠIĆ*****, Ljiljana MOJOVIĆ***
**Innovation Center of the Faculty of Technology and Metallurgy, University of Belgrade, Karnegijeva 4, Belgrade, Serbia*
***Faculty of Technology and Metallurgy, University of Belgrade, Karnegijeva 4, Belgrade, Serbia*
****Faculty of Technology, University of Novi Sad, Bulevar cara Lazara 1, Novi Sad, Serbia*
*****Maize Research Institute Zemun Polje, Slobodana Bajića 1, 11185 Belgrade, Serbia*
*****Institute of Food Technology, University of Novi Sad, Bulevar cara Lazara 1, Novi Sad, Serbia*
kontakt: dmladenovic@tmf.bg.ac.rs

ABSTRACT

Brewer's spent grain (BSG) was used in this study as a support for the immobilization of Lactobacillus paracasei NRRL B-4564, thus enabling the recirculation of immobilized biomass in repeated-batch fermentation. The chemical composition and the energy parameters of the fermented and non-fermented BSG were analyzed and compared. Moreover, the probiotic features of L. paracasei were analyzed to examine the possibility of using fermented BSG as a functional ingredient in ruminant diets.

The results obtained indicate that the fermented BSG had significantly higher protein and ash contents, as well as a significantly lower content of fiber fractions. Furthermore, the fermentation process increased the BSG energy content. The analysis of probiotic potential revealed a high tolerance of L. paracasei to pH 2.5 and bovine bile, autoaggregation ability and antimicrobial activity, suggesting that the fermented BSG with immobilized microbial biomass can be used as functional feed in ruminant diets.

Keywords: *brewer's spent grain, lactic acid, probiotics, ruminant feed*

REZIME

Globalna potražnja za hranom animalnog porekla raste kao posledica kontinuiranog rasta populacije, urbanizacije i porasta prihoda. Kako bi se zadovoljile potrebe tržišta, upotreba nekonvencionalnih hraniva i sporednih agro-industrijskih proizvoda u ishrani životinja postaje uobičajena praksa. U ovom radu je ispitivan uticaj mlečno-kiselinske fermentacije na kvalitet pivskog tropa kao hraniva za preživare. Pivski trop je korišćen kao nosač za imobilizaciju Lactobacillus paracasei NRRL B-4564, što je omogućilo recirkulaciju imobilisane mikrobne biomase u više uzastopnih šaržnih ciklusa. Po završetku poslednje fermentacione šarže, pivski trop sa imobilisanom biomasom je odvojen od fermentacionog medijuma i osušen, nakon čega su ispitivani hemijski sastav i energetski parametri relevantni za njegovu upotrebu u ishrani preživara. Dodatno, analizirana su probiotiska svojstva L. paracasei, kako bi se u potpunosti sagledala mogućnost primene fermentisanog pivskog tropa kao funkcionalnog hraniva.

Utvrđeno je da fermentisani pivski trop ima značajno veći sadržaj proteina i pepela, kao i znatno manji sadržaj svih frakcija vlakana u odnosu na nefermentisane uzorke. Takođe, fermentacija je dovela do povećanja sadržaja energije pivskog tropa. Analizom probiotskih karakteristika, utvrđeno je da L. paracasei ima visoku stopu preživljavanja pri pH 2.5 i u prisustvu goveđe žuči, sposobnost autoagregacije, kao i antimikrobnu aktivnost prema Gram-pozitivnim (Bacillus cereus) i Gram-negativnim (Escherichia coli) patogenim bakterijama.

Na osnovu fenotipskih karakteristika L. paracasei, kao i povoljnog uticaja mlečno-kiselinske fermentacije na kvalitet pivskog tropa, može se zaključiti da se fermentisani pivski trop sa imobilisanom mikrobnom biomasom može koristiti kao funkcionalno hranivo u obrocima namenjenim ishrani preživara.

Ključne reči: *pivski trop, mlečna kiselina, probiotici, hranivo za preživare.*

INTRODUCTION

The global demand for food of animal origin is increasing significantly due to the continued growth of the world population, urbanization, and economy. In the period 2005-2050, the global demand for meat and milk is expected to increase by 57 % and 48 %, respectively *(Mottet et al., 2017)*. Increased livestock production has become a great challenge, particularly for developing countries, imposing the use of different unconventional feed resources in animal nutrition. Using nutrient resources that are inedible for humans is a possible strategy for both reducing the competitiveness between food and feed and mitigating the environmental impacts of livestock production. Moreover, the costs of conventional feeds are very high and account for 60-70 % of the production costs in intensive dairy farming *(Salami et al., 2019)*. Therefore, supplanting common feed ingredients with some alternative feeds could largely contribute to the sustainability of the livestock industry.

A number of production residues and by-products are considered a great source of nutrients in livestock diets *(Čolović et al., 2018; Šćiban et al., 2013; Semenčenko et al., 2014)*. However, the vast majority of these side streams are still underutilized. The impediments for their broader application in animal farming entail the fluctuating nutrient composition and availability, high fiber contents and low digestibility, the presence of anti-nutritional compounds, a relatively short storage life, the need for further processing, and uncertain supplies. Different methods for tackling these obstacles have been

investigated. The fermentation with fungi or yeast *(Shrivastava et al., 2014)*, the supplementation with cellulases and hemicellulases *(Abdel-Aziz et al., 2015; Li et al., 2016)* and using inoculants containing selected strains of lactic acid bacteria (LAB) have shown promising results in improving nutrient content and digestibility of unconventional feeds. In addition to the favorable effects on the feed quality, many LAB are traditionally used as probiotic feed additives either in ruminant or monogastric diets, significantly contributing to host health through various mechanisms. The interest in using probiotics in the livestock industry has increased considerably, especially after the prohibition of antibiotics as growth promoters. A number of studies have shown that probiotics containing LAB prevent acidosis in ruminants fed with high-concentrate diets, increase weight gain, and reduce the incidence of diarrhea *(Abe et al., 2010; Lema et al., 2001; Stover et al., 2015)*.

Using brewer's spent grain (BSG) as a physical support for *Lactobacillus paracasei* NRRL B-4564, we examined the effects of lactic acid fermentation and associated biofilms on the quality of BSG as ruminant feed. The chemical composition and energy values of the fermented and non-fermented BSG were analyzed and compared. Moreover, the probiotic features of *L. paracasei* were assessed to determine the additional value provided by the microbial biomass attached to the BSG surface.

MATERIAL AND METHOD

Lactic acid fermentation with the recirculation of microbial biomass immobilized onto brewer's spent grain

Lactic acid fermentation was performed using a substrate composed of distillery stillage and sugar beet molasses. The distillery stillage and sugar beet molasses used were kindly provided by the Reahem ethanol plant (Reahem d.o.o., Srbobran, Serbia) and the Alpis-SLC ethanol plant (Swan lake d.o.o., Belgrade, Serbia), respectively. The distillery stillage was centrifuged (Sigma® model 2–16P, Osterode am Harz, Germany) and the resulting supernatant (thin stillage) was mixed with molasses in the ratio of 6:1. Dry BSG, provided by Carlsberg Serbia d.o.o., Čelarevo, Serbia, was used as a carrier for cell immobilization. The adsorption of *L. paracasei* NRRL B-4564 onto BSG was performed according to the previously described procedure *(Mladenović et al., 2017)*.

Lactic acid fermentation was conducted in the repeated-batch mode at an agitation speed of 150 rpm and a temperature of 41 °C (KS 4000i control, IKA®, Staufen, Germany), with the adjustment of pH to 6.5. After the sugar content in the media was reduced to less than 15 g/l, the BSG with immobilized biomass was separated by centrifugation and used as an inoculum for initiating the subsequent batch cycle. A total of five fermentation batches were performed. During the fermentation process, the concentration of total sugar and lactic acid and the number of immobilized cells were determined as described by *Mladenović et al., (2017)*.

Analysis of the fermented and non-fermented brewer's spent grain

After the last fermentation batch, the BSG with immobilized biomass was separated by centrifugation and dried in an oven to a constant mass. The total nitrogen content of the samples was estimated using the Kjeldahl method, and the factor of 6.25 was used to calculate the content of crude protein *(AOAC, 2005)*. The sample oil content was determined according to the Soxhlet method, and the sample ash content was estimated by the sample combustion in a muffle furnace at 650 °C *(AOAC, 2005)*. The neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) of the samples were estimated using the Van Soest detergent method *(Goering and Van Soest, 1970)*. The digestible energy at production level of intake ($DE_{3\times}$), metabolizable energy at production level of intake ($ME_{3\times}$), and net energy for lactation at production level of intake ($NEL_{3\times}$) of the samples were calculated using the summative approach of the National Research Council *(NRC, 2001)*. The net energy for maintenance (NEm) and the net energy for growth (NEg) of the samples were calculated using the equations suggested by the *NRC, (1996)*. The non-fermented BSG was analyzed in the same fashion, serving as a control sample.

***In vitro* analysis of the probiotic features of *L. paracasei* NRRL B-4564**

Low pH and bile tolerance

The tolerance of *L. paracasei* NRRL B-4564 to low pH and bovine bile was studied according to the modified method of *Arasu et al., (2014)*. For this purpose, a total of three different MRS broths were prepared. For a low pH tolerance assay, the pH value of MRS broth was adjusted to pH 2.5 by addition of a 2 M HCl solution. For a bile tolerance test, the MRS broth was supplemented with 0.3 % (w/v) bovine bile (Torlak, Serbia), whereas the MRS with pH 6.5 was used as a control. The experiments were performed in 100 ml flasks containing 60 ml of sterile MRS broths, which were inoculated by the overnight culture of *L. paracasei* (5 % v/v). The samples were incubated under microaerophilic conditions at 37 °C for 4 h. The viable cell number at different points of incubation was determined using the pour plate method on MRS agar. The number of colonies was counted after incubation at 37 °C for 48 h. The survival of *L. paracasei* was expressed as a percentage of the control sample.

Antimicrobial activity

L. paracasei was propagated overnight in MRS broth at 37 °C under anaerobic static conditions. The culture was centrifuged (6000×*g*, 10 min), and the supernatant and neutralized supernatant were further subjected to the agar well diffusion assay. Two pathogenic bacteria (namely *Bacillus cereus* ATCC 11778 and *Escherichia coli* ATCC 25922) were used as indicator microorganisms. A ceramic well (5 mm diameter) was placed in a Petri dish, and the soft nutrient agar inoculated with 1 % (v/v) overnight culture of the indicator strain was poured and left to solidify. After removing the well, 100 µl of the cell-free supernatant was added in the hole. To confirm the production of antimicrobial compounds of proteinaceous nature, a small amount of crystalline proteolytic enzyme pronase E (4000 U mg^{-1}, Sigma-Aldrich, USA) was placed close to the edge of well. The Petri dishes were incubated at 37 °C for 12 h. A bright (halo) zone around the well indicated antimicrobial activity, whereas the absence of an inhibition zone around the pronase E confirmed the proteinaceous nature of the inhibitory compound.

Autoaggregation ability

The autoaggregation of *L. paracasei* was determined according to the procedure of *Collado et al., (2008)*. Briefly, the overnight culture of *L. paracasei* was centrifuged (6000×*g*, 10 min), the cells were washed in the sterile PBS buffer (pH 7.2) and resuspended in the same buffer to absorbance (A_{600nm})=0.25±0.25. Consequently, the concentration of cells in suspension was standardized to 10^7-10^8 CFU/ml. The cell suspensions (4 mL) were mixed briefly on a vortex mixer and incubated at 37 °C for 24 h. The autoaggregation process was monitored by measuring the absorbance at 600 nm. The percentage of autoaggregation was calculated as follows: $(1 - A_t/A_{0h}) \times 100$, where A_t is the absorbance at time t, whereas A_{0h} is the absorbance at t = 0h.

Statistical analysis

All the experiments and analytical methods were performed in triplicate. The results were presented as means ± standard deviation. The differences between the means were assessed using a one-way analysis of variance (ANOVA) followed by the Tukey's test. The *p*-values of less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

The quality of brewer's spent grain as animal feed

This study was aimed to investigate the effect of lactic acid fermentation on the nutritional quality of BSG. The fermentation was performed using BSG as a physical support for *L. paracasei*, enabling the recirculation of immobilized biocatalyst in several successive batch cycles. The chemical composition and the energy parameters of the fermented BSG, alongside the immobilized biomass of *L. paracasei*, were assessed relative to their use in ruminant nutrition. The chemical analysis revealed that lactic acid fermentation considerably affected the composition of BSG (Fig. 1). The fermented BSG had significantly higher crude protein and ash contents compared to the non-fermented sample. Simultaneously, the fiber content of the fermented BSG sample was significantly lower after lactic acid fermentation. Among the cell wall fractions, the content of NDF was the most affected, followed by ADL, and ADF. The fermentation did not cause significant changes in the oil concentration of BSG.

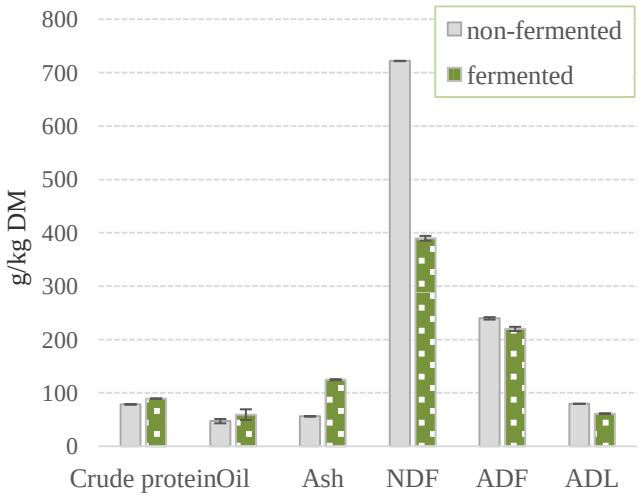

Fig. 1. Chemical composition of the fermented and non-fermented brewer's spent grain

Agro-industrial residues and by-products are often characterized by high fiber and low protein contents, suggesting their poor nutritional quality. Many of these residues are not suitable for monogastric animals and, in some cases, they are not even convenient for ruminant nutrition due to their low digestibility *(Villas-Bôas et al., 2002)*. Therefore, the main challenge of using agro-industrial residues and by-products in animal nutrition is enhancing their nutritional value, digestibility and dry matter intake. The protein content of the fermented BSG was 13.7 % higher than that of the non-fermented BSG sample. In addition, the content of fiber fractions of the fermented BSG sample was decreased by 8.26-46.09 %, indicating that lactic acid fermentation had a very favorable effect on the quality of BSG. *L. paracasei* NRRL B-4564 is not capable of producing cellulolytic enzymes, so the reduction of fiber fractions in BSG was not a result of enzymatic hydrolysis. On the other hand, *L. paracasei* NRRL B-4564 has been proven to be capable of producing lactic acid in high concentrations *(Mladenović et al., 2017)*, indicating that a decrease in fiber content could be a result of acid hydrolysis during repeated batch fermentation. Similar to the findings of this study, *Liu et al., (2015)* reported that treatments of rice straw using LAB improved its feed quality and digestibility, as evidenced by higher crude protein concentrations and lower contents of NDF and ADF.

The energy values of the non-fermented and fermented BSG samples were calculated following the NRC recommendations and nutrient requirements of dairy and beef cattle. It can be seen that the predicted energy parameters were significantly higher after lactic acid fermentation (Fig. 2). The energy values of the feed are related to its chemical characteristics, and in general, these values are lower in the feed having higher fiber contents. In the present study, the reuse of BSG with immobilized biomass in successive fermentation batches led to significant reductions in the fiber content of BSG, consequently improving its energy value.

The number of viable cells per gram of BSG at the end of the fifth fermentation batch was very high and amounted to 5×10^{10} CFU/g, indicating a satisfactory adsorption ability of *L. paracasei* and a stable cell attachment on the BSG surface. In addition to the improved chemical composition and energy content of BSG, the biomass of *L. paracasei* NRRL B-4564 attached to its surface could provide an additional advantage to animal diet. To fully consider the possibility of using fermented BSG as a functional feed ingredient, the probiotic characteristics of *L. paracasei* NRRL B-4564 strain were further assessed.

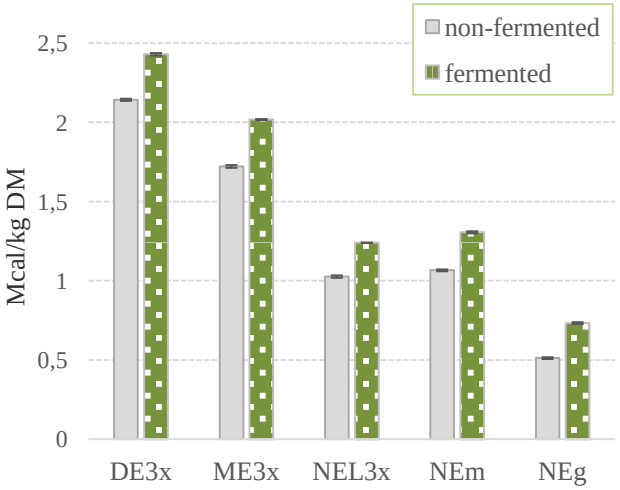

Fig. 2. Energy values of the fermented and non-fermented brewer's spent grain

Probiotic potential of lactic acid-producing strain

In order to provide health benefits when consumed, the probiotic microorganisms should be able to survive the passage through the gastrointestinal tract (GIT), i.e. the acidic environment of the stomach and the presence of bile salts in the small intestine. In an attempt to evaluate the ability of *L. paracasei* NRRL B-4564 to survive the unfavorable environment of the GIT, the strain was exposed to a pH value of 2.5 and a bovine bile concentration of 0.3 %. The results of acid and bile tolerance are presented in Fig. 3. *L. paracasei* exhibited high tolerance to low pH value and bovine bile during 4 h of exposure. After 4 h of incubation, the survival rates of *L. paracasei* at pH 2.5 and 0.3 % of bovine bile were 91 % and 96 % respectively, compared to a control sample. The ability of LAB to survive the conditions of the GIT was reported to be very variable, even within the same bacterial species, indicating

that these characteristics are strain-specific *(Mishra and Prasad, 2005; Ren et al., 2014)*. *Jacobsen et al., (1999)* reported a complete loss of viability for 15 out of the 44 tested strains after 4 h of the exposure to pH 2.5, whereas almost all the examined strains survived the bile salt concentration up to 0.3 %. The high survival rate of *L. paracasei* NRRL B-4564 under the testing conditions of the present study (Fig. 3) indicates that this strain can provide health benefits when consumed, thus confirming its potential as a probiotic additive.

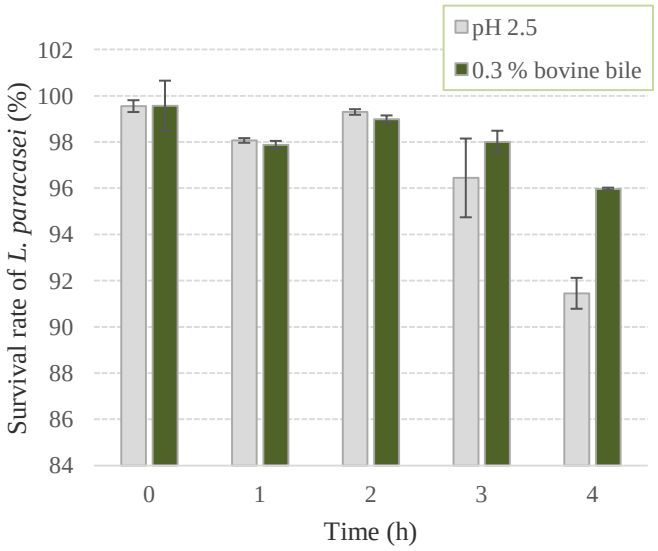

Fig. 3. Survival of L. paracasei NRRL B-4564 at low pH value and in the presence of bovine bile

The ability of bacterial cells to attach or adhere to the intestinal surface is another essential feature of probiotic strains. In many cases, this feature is related to the aggregation ability of a probiotic strain *(Del Re et al., 2000; Pérez et al., 1998)*. The ability of probiotic strains to aggregate is considered a desirable characteristic as such strains can potentially inhibit the binding of pathogenic microorganisms to intestinal surfaces. This can be achieved either by forming a barrier via autoaggregation, coaggregation with commensal microorganisms on the intestinal mucosa, or providing a direct coaggregation with intestinal pathogens, thereby facilitating their removal from GIT. The autoaggregation ability of *L. paracasei* NRRL B-4564 was estimated by measuring the optical density of cell suspensions for 24 h (Fig. 4). It can be observed that the percentage of autoaggregation increased during the incubation period. A very intensive formation of aggregates occurred during the first 3 h when an autoaggregation percentage of 20.7 % was achieved. The percentage of autoaggregation of *L. paracasei* after 24 h of incubation in the PBS buffer (44.1 %) obtained in the present study was close to the values previously reported for *L. casei* (40.4 %), *L. acidophilus* (33.5 %), *L. rhamnosus* (38.7 %) and *L. salivarius* (54.5 %) *(Collado et al., 2008)*. It is noteworthy that high autoaggregation values are not necessarily associated with the *in vivo* adhesion, which involves various host factors such as defense mechanisms, resident (permanent) microbiota and peristaltic movements. These factors can significantly affect and modify bacterial cell adhesion to the intestinal surface *(Verón et al., 2017)*. For the evaluation of the *L. paracasei* antimicrobial activity, the cell-free supernatants were tested against two indicator pathogenic microorganisms using the agar well diffusion method. The results obtained indicate that the cell-free supernatant at the acidic pH inhibited the growth of Gram-positive (*B. cereus*) and Gram-negative bacteria (*E. coli*). The supernatant of *L. paracasei* NRRL B-4564 was not sensitive to pronase E.

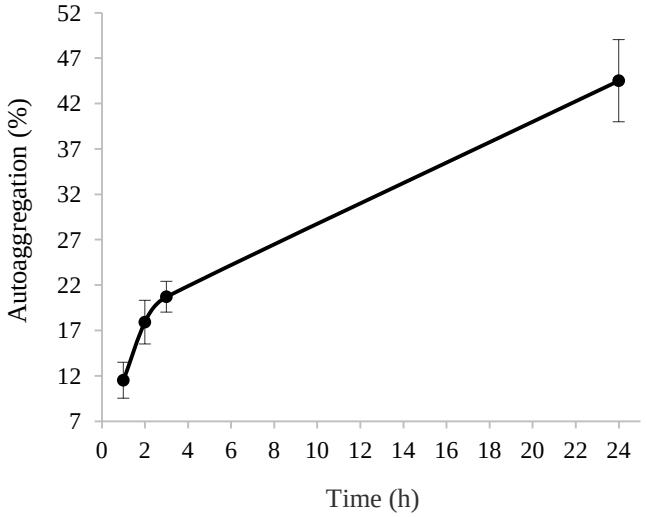

Fig. 4. Autoaggregation of L. paracasei NRRL B-4564 during the incubation in the PBS buffer

Moreover, the absence of an inhibition zone around the well containing the neutralized cell-free supernatant confirmed that the antimicrobial effect was caused by the produced lactic acid and not by the compounds of proteinaceous nature such as bacteriocins. Lactic acid is a major metabolite of many *Lactobacillus* and *Bifidobacterium* species, leading to a decrease in pH and the associated antimicrobial effect *(Tejero-Sariñena et al., 2012)* (which significantly contributes to the safety of fermented products and the stability of the gut microbiota *(Bendali et al., 2011; Cizeikiene et al., 2013))*. The mechanism of the inhibitory effect caused by lactic acid is based on the fact that the undissociated form of acid diffuses through the cell membrane and dissociates within the cell releasing of H^+ ions, which creates an acidic environment within the cell. In addition to the pH effect, undissociated acid disrupts the electrochemical proton gradient, causing bacteriostasis and, ultimately, the death of susceptible bacteria *(Magnusson and Schnurer, 2005)*.

According to the phenotypic characteristics of *L. paracasei* NRRL B-4564 confirmed in this study, the microbial biomass attached to the BSG surface could be of additional value to host health.

CONCLUSIONS

In the present study, the possibility of improving the quality of BSG as an animal feed ingredient was studied. The results obtained indicate that using BSG as a support for *L. paracasei* NRRL B-4564 in repeated-batch fermentation significantly improved its nutrient composition and energy value. The findings obtained on the probiotic features of *L. paracasei* NRRL B-4564 suggest that fermented BSG and the *L. paracasei* biomass attached to its surface can be used as a functional ingredient in ruminant diets.

ACKNOWLEDGMENT: This paper was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project TR 31017 and Project #I-1/2018 of Scientific and Technological Collaboration of the Republic of Serbia and the PR China).

REFERENCES

Abdel-Aziz, N.A., Salem, A.Z.M., El-Adawy, M.M., Camacho, L.M., Kholif, A.E., Elghandour, M.M.Y., Borhami, B.E.,

2015. Biological treatments as a mean to improve feed utilization in agriculture animals-An overview. J. Integr. Agric. 14(3), 534-543.

Abe, F., Ishibashi, N., Shimamura, S., 2010. Effect of Administration of Bifidobacteria and Lactic Acid Bacteria to Newborn Calves and Piglets. J. Dairy Sci. 78, 2838–2846.

Association of Official Analytical Chemists. Official Methods of Analysis, 18th international edition. Methods 923.03, 930.15, 955.04, 960.39. AOAC International, Gaithersburg, MD, 2005.

Arasu, M.V., Kim, D.H., Kim, P. Il, Jung, M.W., Ilavenil, S., Jane, M., Lee, K.D., Al-Dhabi, N.A., Choi, K.C., 2014. In vitro antifungal, probiotic and antioxidant properties of novel *Lactobacillus plantarum* K46 isolated from fermented sesame leaf. Ann. Microbiol. 64, 1333–1346.

Bendali, F., Madi, N., Sadoun, D., 2011. Beneficial effects of a strain of *Lactobacillus paracasei* subsp. *paracasei* in *Staphylococcus aureus*-induced intestinal and colonic injury. Int. J. Infect. Dis. 15, e787–e794.

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, 539–545.

Collado, M.C., Meriluoto, J., Salminen, S., 2008. Adhesion and aggregation properties of probiotic and pathogen strains. Eur. Food Res. Technol. 226, 1065–1073.

Čolović, Dušica, Banjac, V., Rakita, Slađana, Čolović, R., Marjanović-Jeromela, Ana, Vidosavljević, S., Kokić, Bojana, 2018. By-products of black (*Brassica Nigra*) and white (*Sinapis Alba*) mustard seed production as animal feed: Possibilities and hazards. J. Process. Energy Agric. 22, 188–191.

NRC. Nutrient requirements of dairy cattle: 2001, 7th revised ed. National Academies Press, Washington, 2001.

NRC. Nutrient requirements of beef cattle: 1996, 8th revised ed. National Academies Press, Washington, 1996.

Del Re, B., Sgorbati, B., Miglioli, M., Palenzona, D., 2000. Adhesion, autoaggregation and hydrophobicity of 13 strains of *Bifidobacterium longum*. Lett. Appl. Microbiol. 31, 438–442.

Goering HK, Van Soest PJ. Forage fiber analyses: apparatus, reagents, procedures, and some applications. Agricultural Research Service, US Department of Agriculture; 1970.

Jacobsen, C.N., Nielsen, V.R., Hayford, A.E., Møller, P.L., Michaelsen, K.F., Paerregaard, A., Sandström, B., Tvede, M., Jakobsen, M., 1999. Screening of probiotic activities of forty-seven strains of *Lactobacillus* spp. by in vitro techniques and evaluation of the colonization ability of five selected strains in humans. Appl. Environ. Microbiol., 65(11), 4949-4956.

Lema, M., Williams, L., Rao, D.R., 2001. Reduction of fecal shedding of enterohemorrhagic *Escherichia coli* O157:H7 in lambs by feeding microbial feed supplement. Small Rumin. Res. 39, 31–39.

Li, X., Xu, W., Yang, J., Zhao, H., Pan, C., Ding, X., Zhang, Y., 2016. Effects of applying lactic acid bacteria to the fermentation on a mixture of corn steep liquor and air-dried rice straw. Anim. Nutr. 2, 229–233.

Liu, J.J., Liu, X.P., Ren, J.W., Zhao, H.Y., Yuan, X.F., Wang, X.F., Salem, A.Z., Cui, Z.J., 2015. The effects of fermentation and adsorption using lactic acid bacteria culture broth on the feed quality of rice straw. J. Integr. Agric. 14(3), 503-13.

Magnusson, J., Schnurer, J., 2005. Antifungal lactic acid bacteria as bio-preservations. Trends Food Sci. Technol. 16, 70–78.

Mishra, V., Prasad, D.N., 2005. Application of in vitro methods for selection of *Lactobacillus casei* strains as potential probiotics. Int. J. Food Microbiol. 103, 109–115.

Mladenović, Dragana, Djukić-Vuković, Aleksandra, Radosavljević, M., Pejin, Jelena, Kocić-Tanackov, Sunčica, Mojović, Ljiljana, 2017. Sugar beet pulp as a carrier for *Lactobacillus paracasei* in lactic acid fermentation of agro-industrial waste. J. Process. Energy Agric. 21, 41–45.

Mottet, A., Haan, C. de, Falcucci, A., Tempio, G., Opio, C., Global, I., Security, F., 2017. Livestock: On our plates or eating at our table? A new analysis of the feed/food debate. Glob. Food Sec. 14, 1–8.

Pérez, P.F., Minnaard, Y., Disalvo, E.A., De Antoni, G.L., 1998. Surface properties of bifidobacterial strains of human origin. Appl. Environ. Microbiol. 64, 21–26.

Ren, D., Li, C., Qin, Y., Yin, R., Du, S., Ye, F., Liu, C., Liu, H., Wang, M., Li, Y., Sun, Y., Li, X., Tian, M., Jin, N., 2014. Invitro evaluation of the probiotic and functional potential of *Lactobacillus strains* isolated from fermented food and human intestine. Anaerobe 30, 1–10.

Salami, S.A., Luciano, G., O'Grady, M.N., Biondi, L., Newbold, C.J., Kerry, J.P., Priolo, A., 2019. Sustainability of feeding plant by-products: a review of the implications for ruminant meat production. Anim. Feed Sci. Technol. 251, 37-55.

Šćiban, Marina, Kukić Dragana, Ivetić Darjana, Prodanović Jelena, Antov Mirjana., 2013. Possibility of using of treated beet shreds from process of bioethanol production for animal feed. J. Process. Energy Agric 17, (3)124-126.

Semenčenko, Valentina, Radosavljević, Milica, Terzić, Dušanka, Milašinović-Šeremešić, Marija, Mojović, Ljiljana, 2014. Dried distillers' grains with solubles (DDGS) produced from different maize hybrids as animal feed. J. Process. Energy Agric. 18, 80–83.

Shrivastava, B., Jain, K.K., Kalra, A., Kuhad, R.C., 2014. Bioprocessing of wheat straw into nutritionally rich and digested cattle feed. Sci. Rep. 4, 6360.

Tejero-Sariñena, S., Barlow, J., Costabile, A., Gibson, G.R., Rowland, I., 2012. *In vitro* evaluation of the antimicrobial activity of a range of probiotics against pathogens: Evidence for the effects of organic acids. Anaerobe 18, 530–538.

Verón, H.E., Di Risio, H.D., Isla, María Inés, Torres, S., 2017. Isolation and selection of potential probiotic lactic acid bacteria from *Opuntia ficus-indica* fruits that grow in Northwest Argentina. LWT - Food Sci. Technol. 84, 231–240.

Villas-Bôas, S.G., Esposito, E., Mitchell, D.A., 2002. Microbial conversion of lignocellulosic residues for production of animal feeds. Anim. Feed Sci. Technol. 98 (1-2), 1-12.

Stover MG, Watson RR, Collier RJ. Pre-and probiotic supplementation in ruminant livestock production. In: Probiotics, Prebiotics, and Synbiotics: Bioactive Foods in Health Promotion. Elsevier Inc., 2015, pp. 25-36.

Received: 27. 04. 2020. Accepted: 03. 06. 2020.