VETERINARSKI ARHIV 87 (1), 103-112, 2017

The effect of *Lactobacillus sakei* supplementation on milk yield, lipid profile and oxidative status in lactating dairy cows: a preliminary study - short communication

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TVARIJONAVICIUTE, A., I. MONKEVICIENE, E. BARTKIENE, J. PASTOR, R. ZELVYTE: The effect of *Lactobacillus sakei* supplementation on milk yield, lipid profile and oxidative status in lactating dairy cows: a preliminary study - short communication. Vet. arhiv 87, 103-112, 2017.

ABSTRACT

The aim of the study was to investigate if feeding with *Lactobacillus sakei* could be beneficial for dairy cows. For this purpose, twenty mid-lactation cows were randomly allocated into two groups: the control, which received standard food, and the test group, which received standard food with *L. sakei* supplementation ($5.0*10^8$ colony forming units of *L. sakei* per day) for a two month period. Before and after the experimental period milk production and serum lipid profile (total cholesterol, high density lipoprotein cholesterol, and triglycerides) and oxidative markers (paraoxonase 1, total antioxidant capacity [TAC], total oxidant status [TOS], and oxidative stress index [OSI]) were evaluated. After the experimental period the control group showed very a mild, although significant decrease in serum triglycerides, while in the test group significant decreases in serum total cholesterol and TAC, and increases in triglycerides, TOS, and OSI were observed. The results of the present research indicate that *L. sakei* supplementation may not be beneficial for dairy cows, as alterations in lipid profile and increased oxidative stress were observed, without any increase in milk production.

Key words: cow, lactic acid bacteria, probiotics, total antioxidant capacity, total oxidative status

Introduction

In early and peak lactation cows, energy demands for milk production frequently exceed dietary supply, resulting in an increase in fatty acid mobilization from adipose

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ISSN 0372-5480
Printed in Croatia

tissue that, when the metabolic capacity of the liver is exceeded, leads to lipid accumulation in the liver and impaired liver function (ANTONČIĆ-SVETINA et al., 2011; SORDILLO and RAPHAEL, 2013). This situation is frequently associated with health disturbances, such as oxidative stress or inflammation, and production problems (GERLOFF et al., 1986). For this reason, in the last few years probiotics have been gaining attention in the dairy industry for their role in improving feed efficiency, productive performance, and health (YOON and STERN, 1995; BOYD et al., 2011).

Generally, lactic acid bacteria are well known probiotics, and are used as growth promoters to prevent intestinal infections by pathogenic bacteria, decrease stress, stimulate host immune response and increase milk production in cows (YASUDA et al., 2007). *Lactobacillus sakei* (*L. sakei*) is a Gram-positive anaerobic lactic acid bacteria belonging to Lactobacillus genus. *L. sakei* is commonly found living on fresh meat and fish, and is generally used in the food industry for preservation and storage of fresh meats and fish (CHAILLOU et al., 2005). Recently, the beneficial role of *L. sakei* in humans and laboratory animals was described (MAINARDI et al., 2009; WOO et al., 2010). However, no studies were conducted to evaluate the possible effect of *L. sakei* feeding on ruminants.

It was hypothesized that probiotic supplement could maintain a more stable milk yield in mid-lactation cows, since milk yield begins to decline in this lactation period. Thus the aim of this study was to investigate if *L. sakei* feeding could be beneficial in mid-lactation dairy cows. For this reason, the milk production of cows receiving *L. sakei* and controls was evaluated. In addition, lipid profile (total cholesterol, high density cholesterol [HDL-C], and triglycerides) and oxidative markers (paraoxonase 1 [PON1], total antioxidant capacity [TAC], total oxidant status [TOS], and oxidative stress index [OSI]) in the serum of the dairy cows were measured, to evaluate the possible metabolic effects of *L. sakei* supplementation.

Materials and methods

Animal care and procedures were in accordance with the guidelines of the "Requirements for keeping, maintenance and use of animals intended for experimental and other scientific purposes" (2009). Twenty 5-6 years old mid-lactation, 19-26 weeks postpartum, gestating, multiparous Lithuanian Black&White - Holstein, dairy cows were included in this study. All the cows underwent and passed general health examinations monthly throughout, and complete blood count and biochemical profiles, including haptoglobin as a marker of inflammation, were performed at the beginning and the end of the study to confirm there were no apparent abnormalities.

All the cows were randomly subdivided into two groups: the control group (n = 10) and the test group (n = 10). Cows in the control group received standard feed during the two month period, while cows in the test group were fed standard feed with 5.0×10^8

colony forming units of *L. sakei* per day (RAETH-KNIGHT et al., 2007). The ingredients and nutrient composition of the diets fed to the cows are presented in Table 1. Water was available *ad libitum*. No significant differences were found in terms of age, milk production, and body condition score between the groups.

Table 1. Ingredients and nutrient composition of the diets fed to dairy cows

	Weight, kg	
Diet composition	Group A	Group B
Haylage	22.0	22.0
Triticale	7.0	7.0
Urea	0.055	0.055
Mineral supplement	0.200	0.200
L. sakei supplement	-	0.100
Nutrient content		
Dry matter, kg	17.3	17.4
NEL, MJ	114.3	114.9
Crude protein, g	2514.0	2522.0
Crude fibre, kg	3.3	3.4
Usable crude protein, g	2438.0	2450.0
Ruminal ammonia balance, g	12.1	11.7
Ca, g	108.7	108.8
P, g	67.1	67.4
Ca : P	1.62 : 1	1.61 : 1

Group A = control (basal diet); group B = basal diet plus supplement with *L. sakei*; NEL = netto energy for lactation; Ca = Calcium; P = Phosphorus

Blood samples for biochemical analysis were collected from all the cows at the beginning and the end of the experimental period. Collections were made in the morning (after an overnight fast of at least 12 h) from the v. coccygea. Blood samples were then centrifuged at 2000 g for 10 min at room temperature to obtain serum, which was stored in plastic vials at -20 °C until analysis, less than one month after the final sampling.

EA was analysed by measuring the hydrolysis of p-nitrophenyl acetate to p-nitrophenol as described elsewhere (HAAGEN and BROCK, 1992; TVARIJONAVICIUTE et al., 2012). The reaction was monitored at 405 nm. The nonenzymatic hydrolysis of phenyl acetate, which was based on the hydrolysis rate in the absence of sample, was subtracted from the total hydrolysis rate.

TOS was measured as previously described (EREL, 2005). The method is based on the reaction that the ferric ion makes a coloured complex with xylenol orange in an acidic medium. The colour intensity, which can be measured spectrophotometrically at 560 nm and 800 nm, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide.

TAC was determined as described elsewhere (EREL, 2004). The method used was based on 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate) decolourization by antioxidants according to their concentrations and antioxidant capacities. The colour change was measured as a change in light absorbance at 660 nm. For the process, the assay was calibrated with 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid ((R)-(+)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Sigma-Aldrich Co, St Louis, Mo).

The oxidative stress index (OSI) was calculated as the ratio TOS/TAC (ABUELO et al., 2013).

The three assays showed intra- and inter-coefficients of variation below 15%, and the linearity under dilution test resulted in linear regression equations in which the correlation coefficients did not differ from 1, confidence intervals of the slope and intercept included 1 and 0, respectively and Runs test revealed no deviation from linearity ($P>0.1$).

Serum total cholesterol, triglycerides, and HDL-C were analyzed in the automated clinical chemistry analyser (Olympus AU2700, Olympus Diagnostica GmbH) using commercially available assays (Cholesterol, Triglycerides, HDL cholesterol, respectively, Beckman Coulter, Inc. Ireland) following the manufacturer's indications.

Statistical analysis. All data are presented as median and interquartile ranges. The D'Agostino & Pearson omnibus normality test was performed to assess the normality of data, giving a non-parametric distribution; The Wilcoxon matched-pairs signed rank test was used to compare values before and after the experimental period within the groups, and the Mann Whitney test was used to compare values before and after the experimental period between the groups (Graph Pad Prism Version 5 for Windows, Graph Pad software Inc). Statistical significance was defined as $P<0.05$ on two-tailed testing.

Results

As was expected due to the lactation period of the cows, milk production decreased in both groups of cows ($P<0.01$ in both cases) (Fig. 1) after the experimental period. However, no statistically significant differences in milk production were observed between the two groups of cows before or after the experimental period.

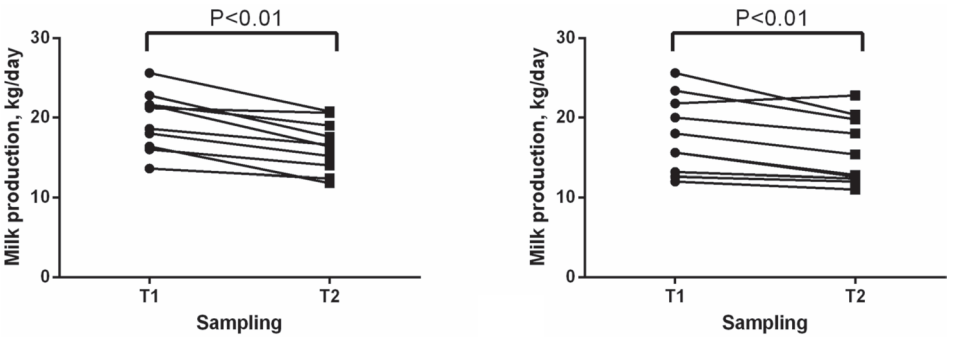

Fig. 1. Milk production in control (a) and test (b) groups of cows before (T1) and after (T2) experimental period

At the beginning of the study no statistically significant differences were observed in any of the evaluated analytes between the two groups (P>0.1 in all cases).

Data on total cholesterol, triglycerides, HDL-C, PON1, TAC, and TOS before and after experimental trial in the two groups of cows are presented in Figs 2 and 3.

The control group showed a very mild, although statistically significant decrease in serum triglyceride concentrations before *vs.* after experimental period, 7.0 (5.3 - 8.1) mg/dL *vs.* 5.2 (3.2 - 6.8) mg/dL.

In the test group statistically significant decreases in serum total cholesterol [149.6 (93.6 - 182.5) mg/dL *vs.* 93.6 (90.7 - 151.8) mg/dL] and TAC concentrations [0.278 (0.264 - 0.311) mmol/L *vs.* 0.252 (0.264 - 0.279) mmol/L], and increases in triglycerides [6.1 (4.9 - 8.5) mg/dL *vs.* 15.3 (8.7 - 18.9) mg/dL], and TOS [3.5 (2.5 - 5.7) µmol/L *vs.* 8.4 (5.5 - 11.0) µmol/L], and OSI [13.2 (8.3 - 22.2) *vs.* 29.9 (21.2 - 46.6)] were observed after the experimental period.

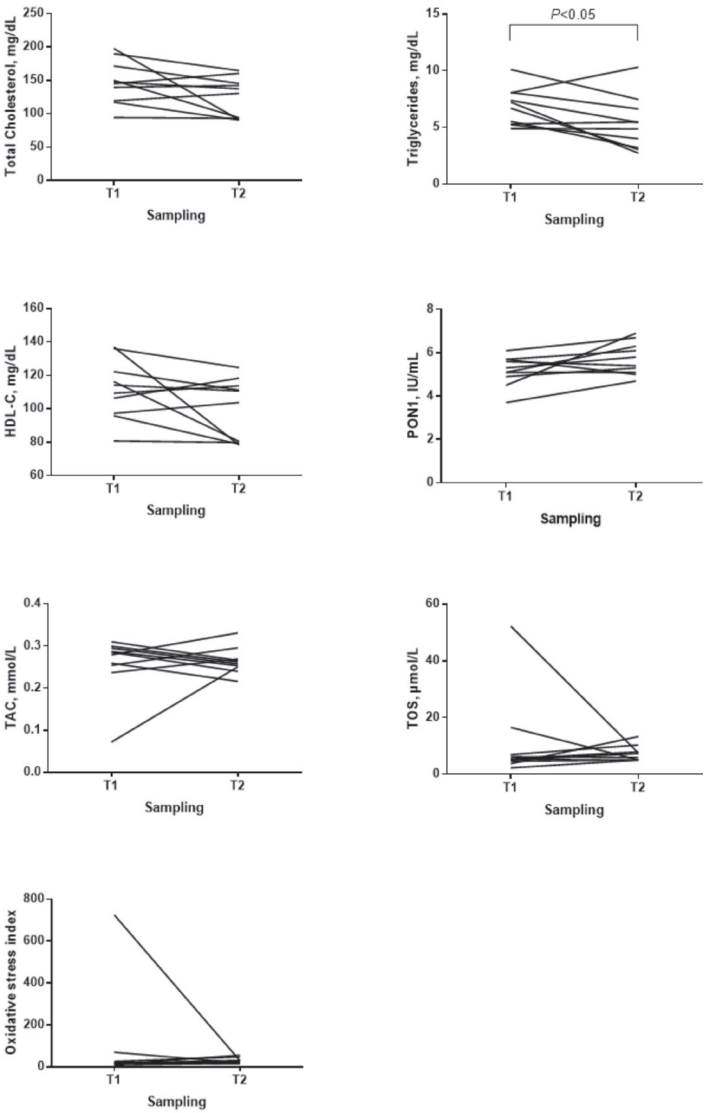

Fig. 2. Total cholesterol, triglycerides, high density lipopoprotein cholesterol (HDL-C), paraoxonase 1 (PON1), total antioxidative capacity (TAC), total oxidant status (TOS), and oxidative stress index (OSI) in control groups of cows before (T1) experimental period

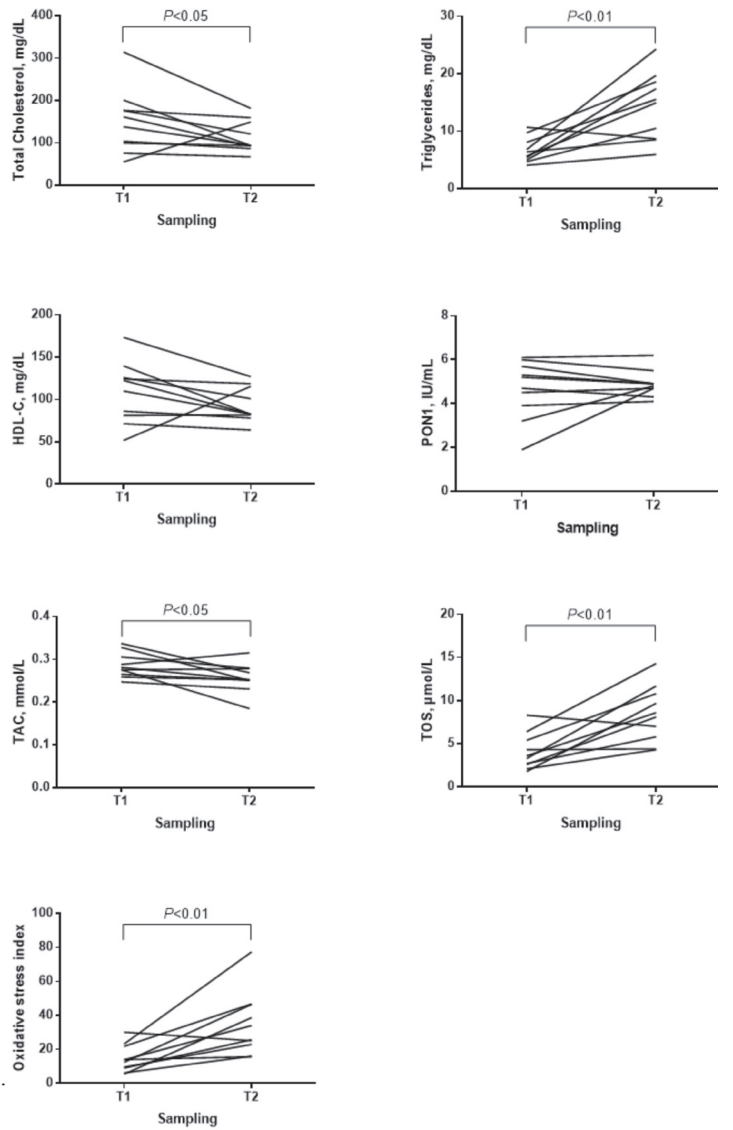

Fig. 3. Total cholesterol, triglycerides, high density lipopoprotein cholesterol (HDL-C), paraoxonase 1 (PON1), total antioxidative capacity (TAC), total oxidant status (TOS), and oxidative stress index (OSI) in control before (T1) and after (T2) experimental period

Discussion

The hypothesis of the present study was that use of *L. sakei* as a feed supplementation could be beneficial for dairy cows and, thus, could be used as a probiotic in this species. The observed data seems to reveal the opposite situation, since *L. sakei* supplementation did not show significant effect on milk production, and resulted in altered lipid profile (decreased total cholesterol and increased triglycerides) and increased oxidative stress (decreased TAC and increased TOS and OSI) in the dairy cows. Lipid mobilization is rare in mid-lactating cows and until the liver metabolic capacity is exhausted, no association with pathologies would be expected (SORDILLO and RAPHAEL, 2013). However, the changes observed in the serum of cows supplemented with *L. sakei* in the present study could be related to the initiation of metabolic pathologies associated with alterations in lipidic profile and oxidative stress as has been described previously, for instance in hepatic lipidosis cases (FARID et al., 2013). For this reason *L. sakei* per os should not be used in this species.

However, these data should be considered with caution because of several limitations. First, a small number of animals was included. Secondly, ideally concentrations of non-esterified fatty acids (NEFAs) should have also been evaluated for lipid metabolism assessment, since NEFAs were described to be more accurate and relevant in assessing lipolysis in ruminants than total cholesterol and triglycerides. Further, although this was performed following the previously described methods, where a sole dosage of probiotics were used (YASUDA et al., 2007; PENHA et al., 2011), ideally more than one dosage of the *L. sakei* supplementation should have been investigated. Finally, in order to clarify the possible causes of these findings, a microbiological study of rumen content should have been performed, evaluating the interactions between *L. sakei* and rumen microflora. Because of these limitations this study should be considered as preliminary.

Conclusion

The results of the present research suggest that *L. sakei* supplementation may not be beneficial for dairy cows, as alterations in circulating lipid profile (decreased serum total cholesterol and increased in triglycerides) and an increase in pro-oxidant status (decreased total antioxidants and increased total oxidants) without any increase in milk production were observed in the studied animals.

Acknowledgements

The authors wish to thank Isabel M. Rodrigez-Muñoz, Josefa Hernandez-Ruiz, and Susana Ros-Lara, University of Murcia, for their technical support. Financial support was provided by the Program "Juan de la Cierva" of 'Ministerio de Economia y Competitividad', Spain, through a postdoctoral grant.

Conflict of interest

None of the authors has any financial or other relationship with other people or organizations that may inappropriately influence this work.

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Received: 8 October 2015
Accepted: 29 September 2016

TVARIJONAVICIUTE, A., I. MONKEVICIENE, E. BARTKIENE, J. PASTOR, R. ZELVYTE: Učinak dodatka *Lactobacillus sakei* na prinos mlijeka, lipidni profil i oksidativni status krava u laktaciji. Vet. arhiv 87, 103-112, 2017.

SAŽETAK

Cilj je ovog rada bio istražiti može li *Lactobacillus sakei* dodan u hranu poboljšati proizvodnju mlijeka u mliječnih krava. U tu je svrhu 20 krava u sredini laktacije nasumično bilo podijeljeno u dvije skupine: kontrolnu, koja je dobivala uobičajenu hranu i pokusnu, koja je dobivala uobičajenu hranu s dodatkom *L. sakei* ($5{,}0x10^8$ stanica *L. sakei* na dan). Pokus je trajao dva mjeseca. Prije i nakon pokusnog razdoblja promatrana je proizvodnja mlijeka i određivan lipidni profil (ukupni kolesterol, kolesterol visoke gustoće i trigliceridi) te oksidacijski biljezi (paraoksonaza 1, ukupni antioksidacijski kapacitet, ukupni oksidacijski status i indeks oksidacijskog stresa). Nakon pokusnog razdoblja kontrolna skupina pokazivala je blago, ali ipak signifikantno sniženje serumskih triglicerida, dok se u pokusne skupine značajno snizio ukupni kolesterol u serumu i ukupni antioksidacijski kapacitet, a povisili su se trigliceridi, vrijednosti ukupnog oksidacijskog statusa i indeks oksidacijskog stresa. Rezultati upućuju na zaključak da dodatak *L. sakei* nije bio od koristi za mliječne krave s obzirom na to da su bile ustanovljene promjene u lipidnom profilu i povišenje oksidacijskog stresa bez povećane proizvodnje mlijeka.

Ključne riječi: krave, bakterije mliječne kiseline, probiotici, ukupni antioksidacijski kapacitet, ukupni oksidacijski status