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The influence of probiotic administration on the phagocytic and oxidative burst activity of neutrophils and monocytes in the peripheral blood of dairy cows during different lactation periods

Piotr Brodzki[1,✉], Hubert Gorzkoś[2], Jan Marczuk[3],
Urszula Lisiecka[4], Andrzej Junkuszew[5], Leszek Krakowski[1],
Marek Szczubiał[1], Nikodem Brodzki[6], Katarzyna Głodkowska[1]

[1]Department and Clinic of Animal Reproduction, [3]Department and Clinic of Animal Internal Medicine,
[4]Department of Epizootiology and Clinic of Infectious Diseases, [6]Faculty of Veterinary Medicine,
University of Life Sciences in Lublin, 20-612 Lublin, Poland
[2]Private Veterinary Practice, Gorvet, 09-227 Sztutowo, Poland
[5]Department of Breeding of Small Ruminants, Faculty of Biology,
Animal Sciences and Bioeconomy, University of Life Sciences in Lublin, 20-028 Lublin, Poland
wetdoc@interia.pl

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Abstract

Introduction: The objective of this bovine peripheral blood study was a comparative assessment of the phagocytic activity of neutrophils and monocytes and of the intracellular killing capacity of neutrophils from cows given no probiotic and from cows which were administered a probiotic consisting of *Saccharomyces cerevisiae*, *Lactobacillus acidophilus*, *Lactobacillus plantarum* and *Rhodopseudomonas palustris*. These activity types were compared during different lactation periods. **Material and Methods:** A cohort of 20 pregnant dairy cows was divided into two groups of 10. The experimental group consisted of cows fed a ration supplemented with probiotics, and the control group consisted of cows fed an unsupplemented ration. Blood was drawn six times: 7 days before dry off, 14 days before parturition, and 7, 21, 60 and 90 days postpartum (DPP). The phagocytic activity of neutrophils and monocytes and the oxidative burst activity of neutrophils were determined by flow cytometry in the peripheral blood of all examined cows. **Results:** Phagocytosis testing revealed increased percentages of phagocytic neutrophils and monocytes in the experimental group at 21, 60 and 90 DPP (P-value < 0.01). The mean fluorescence intensity (MFI) values for neutrophils and monocytes were higher on all days of the study (P-value < 0.01). In oxidative burst testing, the percentages of detected neutrophils and their MFI were increased in the experimental group on all days (P-value < 0.01). **Conclusion:** The use of probiotics supported dairy cows' immunity throughout the whole experiment. Probiotic supplementation may limit the occurrence of infectious diseases in these animals.

Keywords: dairy cows, neutrophils, monocytes, oxidative burst, phagocytosis.

Introduction

Increased productivity and milk yield improvement is constantly striven for in dairy cattle farming. The available literature is dominated by research on increasing milk production through the selection of high-production breeds or provision of health-benefitting balanced feeds and nutritional additives (26, 27). The health of cows is determined by numerous factors, both genetic and environmental. It appears that the perinatal period plays a fundamental role in determining the cow's health and milk yield in the entire lactation period. The perinatal period includes the last weeks of the dry period and the time up to approximately 60 days after delivery, a particularly important phase being the transition period (from three weeks before parturition to approximately three weeks after it). It is the time when intense physiological and hormonal changes occur in dairy cows (9, 11, 41). Numerous studies have confirmed that the perinatal period is the time when dairy cows suffer the worst metabolic and deficiency diseases, such as postpartum hypocalcaemia, hypomagnesaemia, fatty

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liver, ketosis, mastitis, metritis, and displacement of the abomasum (20, 21, 22, 30). Moreover, temporary impairment of the immune functions (immunosuppression) occurs in dairy cows in this period (15, 22). Some authors believe that this immunosuppression in dairy cows in the peripartum period is closely linked to these metabolic diseases or predisposes cows to mastitis, placental retention and metritis. Knowledge of the dynamics and pathophysiology of the immunosuppression observed in this period remains unsatisfactory, although it is constantly expanding. Thus far, no efficient strategies have been formed to improve the defence mechanisms, health and well-being of cows in this critical period, or in other lactation periods (1).

Over the last few years, attention has been paid to the use of probiotics for milk yield improvement and enhancement of cows' health. They contribute to stabilising the balance of the microorganism population and enzymatic activity in the gastrointestinal tract, thus benefitting the growth, development and productivity of the animals. Probiotics are understood to be products containing live or dead microorganisms or substances produced by them, and are typically natural strains of intestinal bacteria specific to the gastrointestinal tract of the given animal species. These bacteria populate the intestines following oral administration and prevent excessive development of pathogenic microorganisms, ensuring better digestion and optimum food use (2, 8, 31). Probiotics may contain one or several strains of microorganism and can be administered in the form of microbial additives, separately or mixed with other substances (33). The mechanisms of action of probiotic microorganisms introduced to the gastrointestinal tract of an animal are primarily competition for adhesion on the intestinal epithelium, competition for nutrients, production of bacteriostatic substances, inhibition of the development of pathogens, and stimulation of systemic immunity (25, 40). The exact mechanism of the immunomodulatory action of probiotics has not been fully understood. It has been demonstrated for humans and mice that complexes of whole probiotic bacteria cells or their fragments with cells of the gut-associated lymphoid tissue have a modulatory impact on T and B lymphocyte function, producing an immune response to antigens. Probiotics also stimulate immunocompetent cells to produce cytokines responsible for intensification, suppression and regulation of systemic and local immune responses (18). Thus far, the impact of probiotic administration on the immune system function in dairy cows remains to be elucidated. The majority of publications (mostly review articles) confirm that probiotics have a stimulating effect on the immunity of cows (4, 38).

The objective of the study was a comparative assessment of the phagocytic activity of neutrophils and monocytes and of the intracellular killing capacity of neutrophils in the peripheral blood of cows. The comparisons were between samples from cows administered the probiotic as a nutritional additive and samples from cows fed without the probiotic addition, and between samples drawn in different lactation periods.

Material and Methods

Experimental animals. The study was approved by the Local Ethics Committee at the University of Life Sciences in Lublin (approval no. 41/2014). The examinations were performed in a herd of 60 dairy Holstein-Friesian cows which were in different stages of lactation. Their milk yields for the 305-day lactation period ranged from 7,200 to 8,720 kg per cow. The cows were kept in a mixed system, chained in position during feeding and milking, and for the rest of the time in a free-range system. Nutrition was based on the total mixed ration (TMR) system. Entirely mixed complete fodder was provided that had a complete nutritional composition adapted to the physiological requirements of the cows. Fodder composition on the farms covered by the experiment was balanced for lactating cows with average milk production of approximately 20 kg. Each cow with a milk yield that exceeded 20 kg received an extra 1 kg of concentrate for every 2 kg of additionally produced milk. The detailed composition of the TMR is presented in Table 1.

Table 1. Composition of the total mixed ration (TMR) and dry mass (DM) daily feed ration for lactating cows

Dose component	Amount per cow, daily	
	kg TMR	kg DM
Maize silage	25.0	8.8
Haylage	8.0	3.2
Ensiled brewery spent grain	8.0	2.7
Wheat straw	0.8	0.7
Ensiled maize grain	2.5	1.7
Ground barley grain	1.5	1.3
Ground triticale grain	1.5	1.3
Ground rapeseed	2.7	2.2
Extracted soyabean meal	2.0	1.7
Glycerine	0.3	0.24
Vitamin and mineral mixture	0.2	0.18
Sodium bicarbonate	0.2	0.2
Calcium carbonate	0.05	0.05
Total	52.75	24.27

The minerals in the vitamin and mineral mixture consisted of calcium carbonate, sodium chloride, sodium phosphate, calcium phosphate, magnesium oxide and magnesium sulphate (23% calcium, 2.2% phosphorus, 9% sodium and 4.5% magnesium). A kilogram of concentrate had the following functional ingredients: 450,000 IU of vitamin A, 45,000 IU of vitamin D_3, 6,000 mg of vitamin E, 400 mg of vitamin K, 1,000 mg of vitamin C, 120 mg of vitamin B_1, 60 mg of vitamin B_2, 30 mg of vitamin B_6, 300 μg of vitamin B_{12}, 6,000 mg of nicotinic acid, 120 mg of pantothenic acid, 75,000 μg of biotin, 6,000 mg of choline chloride, 6,000 mg of zinc, 4,000 mg of manganese, 1,200 mg of copper, 120 mg of iodine, 40 mg of cobalt and 20 mg of

selenium. Nutrition provision was adjusted to actual milk productivity and gestation period; therefore, the proportional composition of the food given above was changed depending on the lactation period of the cows. Rectal examination and ultrasonography of the reproductive system was conducted regularly at monthly intervals. In cows that had no complications during parturition and no signs of inflammation, a protocol synchronising oestrus and ovulation (a presynch-ovsynch protocol) and artificial insemination (AI) with frozen semen were applied. However, cows with detected uterine inflammation were treated for the condition and only subsequently subjected to the synchronisation protocol and AI. The cows with ovarian cycle disturbances were treated individually as necessary for the recognised cause.

Twenty pregnant cows aged 3–5 years were selected for peripheral blood investigation. All selected cows were in the last stage of their lactation period, before the dry period. Their body condition was good and their body condition scores ranged from 3.0 to 3.5. The study included the assessment of the clinical health status of the animals and parasitological examination, which confirmed that all cows were healthy. The selected animals were divided into two groups of 10, only one of which received the probiotic, this group being the experimental group. A 1 mL volume of the probiotic preparation contained 5×10^3 colony-forming units (CFU) of *Saccharomyces cerevisiae*, 5×10^6 CFU of *Lactobacillus acidophilus* and *Lactobacillus plantarum* and *Rhodopseudomonas palustris* and cane molasses. The product was administered in liquid form, as an additive to the standard feed. It was used at the dose recommended by the manufacturer (200 mL of product per cow/day), starting from the period before drying and continuing to the 12th week after birth (90 days postpartum). The probiotic was administered to each cow individually by pouring it over the given allocation of TMR. The control group consisted of cows that were not administered any medicines throughout the experiment, and only the study material was collected from these animals. These cows were fed identically to the experimental group but without the addition of the probiotic. Cows from both groups were subjected to identical research procedures.

Material for cytometric analysis. The test material was peripheral blood that was obtained six times, the first time being on the day the animals were selected. It was drawn 7 days before drying (DBD) and then 14 days before parturition (DBP), 7 days postpartum (DPP), 21 DPP, 60 DPP and 90 DPP. Blood samples in 9 mL volumes were collected from the external jugular vein in ethylenediaminetetraacetic acid or heparinised tubes (Vacutest Kima, Arzergrande (PD), Italy). In 7 DBD, blood was collected for testing before probiotic administration, and after obtaining the material, probiotic administration was started. The biological material collected for laboratory tests was sent to the laboratory within an hour.

Phagocytic activity of neutrophils and monocytes. Neutrophil and monocyte phagocytic activity as the percentage of neutrophils and monocytes that had engulfed bacteria was determined using a commercial Phagotest kit (Orpegen Pharma, Heidelberg, Germany). The tests were performed according to the manufacturer's instructions. The mean fluorescence intensity (MFI) of the phagocytosing cell population was assessed to estimate the individual cellular phagocytic activity as the number of bacteria per cell. Samples of 100 µL of blood were cooled in an ice bath for 15 min, mixed with 2×10^7 of fluorescein isothiocyanate–labelled opsonised *E. coli* which was a component of the Phagotest kit, and then put in a thermostatic chamber at 37°C for 10 min. The control samples were put into an ice bath to inhibit phagocytosis. Afterwards, 100 µL of brilliant blue was added as a quenching solution to suppress the fluorescence of bacteria connected to the leukocyte surface. After two washing steps with 2 mL of washing solution and centrifugation at $2000 \times g$, the supernatant was discarded. Erythrocytes were dissolved using a lysis solution for 20 min at room temperature. As the final step, 50 µL of propidium iodide was added to exclude aggregation artefacts of bacteria or cells (stained leukocytes and bacterial DNA). The tests were performed following the protocol of Panasiuk *et al.* (26).

Oxidative burst. Neutrophil oxidative burst was determined quantitatively with a Bursttest Kit (Orpegen Pharma). Fresh heparinised blood was put in a water bath for 15 min. Then, four test-tubes were filled with 100 µL of blood each and 2×10^7 of unlabelled opsonised *E. coli*, 20 µL of substrate solution as a negative control, 20 µL of peptide *N*-formyl-MetLeuPhe as a chemotactic low physiological stimulus and low control, and 20 µL of phorbol 12-myristate 13-acetate as a strong non-receptor activator and high control. All the samples were incubated at 37°C for 10 min in a water bath. Then, dihydrorhodamine 123 (DHR 123) was added as a fluorogenic substrate and the mixture was incubated again in the same conditions. The oxidative burst occurred with the production of reactive oxygen substrates (ROS) (superoxide anion and hydrogen peroxide) in neutrophils stimulated *in vitro*. In ROS-stimulated neutrophils, nonfluorescent DHR 123 underwent conversion to fluorescent rhodamine 123 (R 123) and the fluorescence was registered in an EPICS XL flow cytometer (Beckman Coulter, Miami, FL, USA). Erythrocytes were removed using a lysing solution for 20 min at room temperature, the solution was then centrifuged for 5 min at $2{,}500 \times g$ and 4°C, and the supernatant was discarded. The samples were washed again with washing solution, centrifuged again for 5 min at $2{,}500 \times g$ and 4°C, and the supernatant was decanted. A 200 µL volume of DNA staining solution was added and the mixture was centrifuged and incubated at 0°C for 10 min in a dark place to discriminate and exclude aggregation artefacts of bacteria and/or cells in flow cytometric analysis. Tests were performed according to the manufacturer's

instructions and the protocol of Panasiuk *et al.* (26) as previously.

Cytometric analysis. The EPICS XL flow cytometer equipped with a 488 nm argon-ion laser was used. The apparatus was calibrated using Flow-Check Fluorospheres (Beckman Coulter). Neutrophil populations were identified by the use of forward and right-angle light scatter. Fluorescence measurements were conducted with identical settings to those for the standard determination of cell phenotype with a fluorochrome-stained monoclonal antibody. Phagocytic activity was determined as the percentage of phagocytising neutrophils and monocytes ingesting one or more bacteria and as the MFI, which was proportional to the mean number of bacteria phagocytised by the cells. Neutrophil oxygen metabolism was determined from the percentage of cells phagocytising *E. coli* producing reactive oxidants (cells undergoing bursts and the change from DHR 123 to R 123), and from the evaluation of neutrophil enzymatic activity (the amount of released active oxygen compounds – the amount of R 123 per cell).

Statistical analysis. All values are presented as means ± standard error of the mean. Statistical analysis was performed using Statistica software version 10.0 (StatSoft, Tulsa, OK, USA, now TIBCO, Palo Alto, CA, USA) and one-way analysis of variance (ANOVA). The results obtained were compared between the control and experimental groups to determine statistical significance using Student's *t*-test, allowing a probability P-value < 0.05 to indicate significance. Statistical differences between the results for the material collected at different times in the group were calculated using ANOVA and the Tukey and Duncan post-hoc tests, again allowing a probability P-value < 0.01.

Results

The percentage of phagocytic neutrophils in the experimental group was significantly lower on the 14th DBP compared to other testing days. This percentage was also lower in comparison to the control group (P-value < 0.001). The values in the experimental group on the 21st, 60th and 90th DPP were significantly higher than those in the control group (P-value < 0.001) (Fig. 1A). The percentage of phagocytic monocytes in both groups of cows was the lowest on the 21st DPP (68.0 ± 3.0 in the experimental group and 44.3 ± 5.1 in the control group) and was significantly so (P-value < 0.01). The values obtained for the experimental group on all testing days were significantly higher than those for the control group (P-value < 0.001) (Fig. 1C). The MFIs of neutrophils and monocytes obtained in the experimental group were lower than those in the control group only on the 7th DBD (P-value < 0.01), and on all remaining testing days were higher (P-value < 0.01) (Fig. 1B and D).

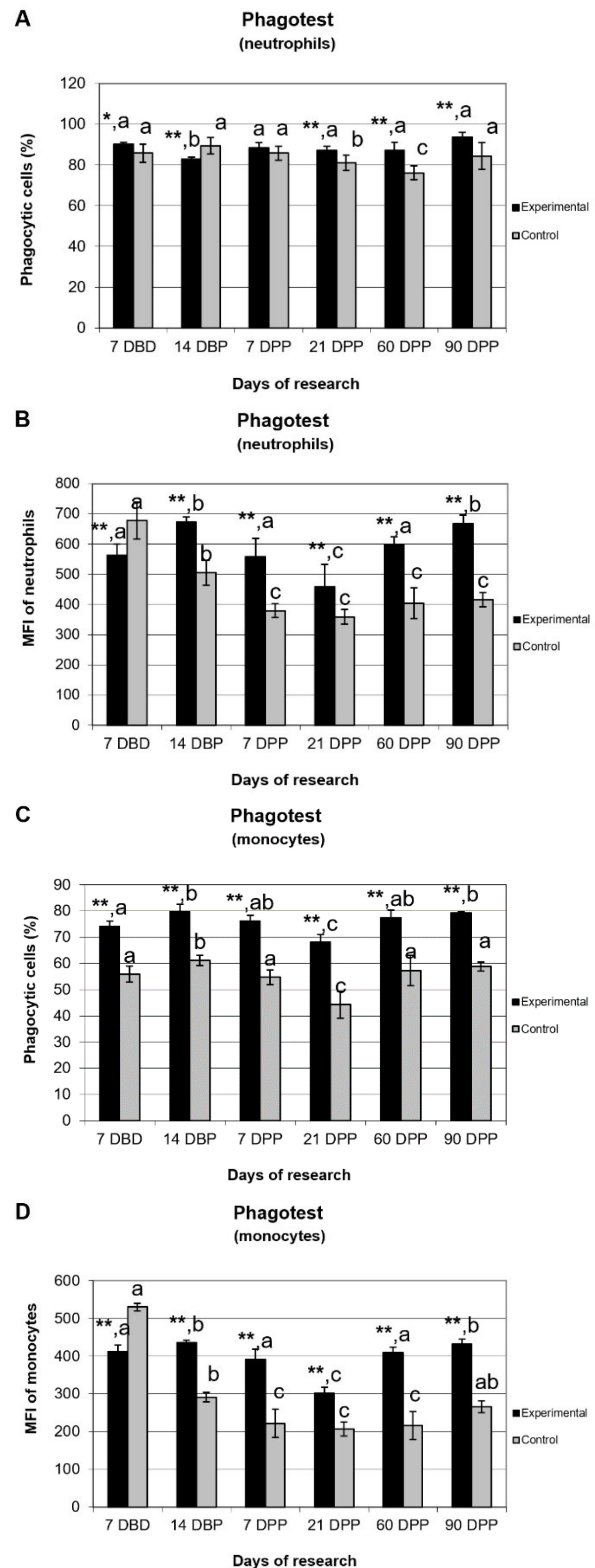

Fig. 1. Cytometric analysis of phagocytosis activity of neutrophils (A and B) and monocytes (C and D) in the peripheral blood of cows at various periods of lactation (mean ± standard error of the mean). DBD – day before drying; DBP – day before parturition; DPP – day postpartum; Experimental – cows provided with probiotic (n = 10); Control – cows without probiotic (n = 10); Phagocytic cells (%) – percentage of neutrophils and monocytes that had engulfed bacteria; MFI – mean fluorescence intensities expressing individual cellular phagocytic activity (number of bacteria per cell). Statistical significance – * p ≤ 0.05; ** p ≤ 0.01 with respect to the control; a–c – significant differences between the results for the material collected at different times in the group (P-value < 0.01)

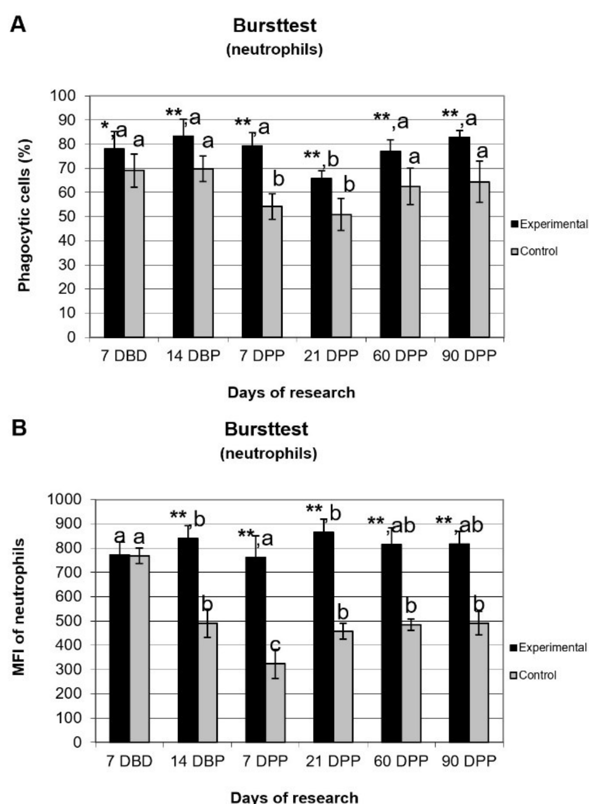

Fig. 2. Cytometric analysis of oxidative burst activity of neutrophils (A and B) in the peripheral blood of cows at various periods of lactation (mean ± standard error of the mean). DBD – day before drying; DBP – day before parturition; DPP – day postpartum; Experimental – cows provided with probiotic (n = 10); Control – cows without probiotic (n = 10); Phagocytic cells (%) – the percentage of phagocytic cells that produced reactive oxygen substrates (measured by the conversion of DHR123 to R123); MFI – mean fluorescence intensities expressing enzymatic activity of phagocytic cells (measured by the amount of R123 per cell). Statistical significance – * $p \leq 0.05$; ** $p \leq 0.01$ with respect to the control; a–c – differences between the results for the material collected at different times in the group (P-value < 0.01)

In the intracellular killing test (the Bursttest), the percentage values of phagocytic cells as well as the MFI of neutrophils were higher in the experimental group than in the control group on all days (P-value < 0.001). The MFIs in both groups were similar only on the first day of study (the 7th DBD) (Fig. 2A and B).

Discussion

The present study assessed selected indices of the systemic immune response in cows during the period from the last week of the previous lactation, through the dry and peripartum periods up to the peak of the subsequent lactation (*i.e.* 90 DPP). The experiment was carried out to determine whether the probiotic nutritional additive influenced the function of the immune system in dairy cows at different stages of lactation. In the literature, there are few studies describing the impact of probiotics on the specific parameters of the immune system in dairy cows. The majority of studies covering cattle contain only general statements that probiotics possess an immunomodulatory effect in this animal species. Furthermore, most dairy cow research undertaken concerning probiotics and the immune system investigated probiotics only used for a restricted time, and maintained observation for a short period, often limited to the transition period (4, 29). This is the most important stage of the entire lactation, since cows must adapt to advanced metabolic transformations caused by parturition and the commencement of milk production (9, 11, 36). However, change in the feeding conditions in dairy cows during the entire lactation occurs several times. The first change takes place before the drying period, another does during the transition period, and yet another also after delivery, during the adjustment of the feed ratio to the current milk yield of the cow. Therefore, according to the authors, knowledge of the outcomes of the application of probiotics in different lactation periods is important, because it enables herd owners to decide whether probiotic administration is reasonable in terms of supporting the activity of the immune system. Perhaps it is sufficient to limit their application to the transition period.

As the present study results indicated, both the phagocytic activity and the intracellular killing capacity of phagocytic cells before probiotic administration (before the drying period) were similar in both groups of cows. The MFI values of neutrophils and monocytes were lower in the experimental group than in the control group. After four weeks of probiotic administration (at 14 DBP), the assessed immune parameters were higher in the experimental group than before administration and were higher than the parameters in the control group, remaining so until the end of the study. This finding is important, as it allows us to assume that cows supplemented with the probiotic had better immune status before parturition and were more capable of adapting in the postpartum period. According to the available literature, this is the period when cows' immune response deteriorates, and thus they are more susceptible to infections, primarily of the uterus and udder (21, 22, 30). The causes of weaker immunity in this period are not entirely understood; however, studies conducted by some authors have demonstrated that elevated levels of non-esterified fatty acids (NEFA) in the blood of a cow during peripartum negative energy balance (NEB) may contribute to immunosuppression (9, 10, 39). It was further demonstrated that a ketone concentration similar to that observed in the peripartum period diminished the phagocytic and killing capacity of neutrophils (PMN) *in vitro* (10). Similarly, high concentrations of NEFA had a negative impact on bovine PMN activity *in vitro* (10, 32). The mechanism by which NEFA negatively affects the function of phagocytic cells is unknown. According to the study presented by Ingvartsen and Moyes (12), fatty acids may also have a supportive effect on the activity of phagocytic cells, depending on the type of fatty acids created in the cow. The present study did not cover NEFA or β-hydroxybutyrate acid (BHBA) level

assessment; however, it cannot be excluded that the concentration of these metabolites was elevated in the studied cows. It appears likely, because on the 21st DPP the assessed immune parameters were observed to be weaker in both groups of cows, but were approximately twice as high in the experimental cows as in the control cows. Furthermore, on the last day of the study, all the assessed parameters were stronger than the baseline values in the experimental cows, whereas in the control group they were mostly weaker. This may indicate that in the cows supplemented with the probiotics, better immune status was maintained throughout the lactation period and these animals had lower susceptibility to infections. However, it is unknown whether this can be linked to the lower content of the previously mentioned NEB markers. The study presented by Nocek *et al.* (24) seems to confirm these presumptions, as it demonstrated lower NEFA concentration in cows fed with fodder containing *Enterococcus faecium*, another probiotic. Similar results were obtained by other authors after the application of *Bacillus subtilis natto* as a nutritional supplement (27). The study of Nocek and Kautz (23) showed that cows supplemented with *Enterococcus faecium* had a lower BHBA concentration in the postpartum period. Also, Luan *et al.* (17) observed decreased ketone concentration after parturition in cows administered *Bacillus pumilus* as a nutritional supplement. Lower NEFA and ketone concentrations indicate that cows mobilise less energy from adipose tissue during periods of high energy demand at the beginning of lactation. Further evidence of a probiotic contribution to energy metabolism efficiency was the increased glucose and insulin concentrations observed in the blood serum of cows following dietary addition of *Enterococcus faecium* in the postpartum period, in contrast to animals not administered this probiotic (27).

The present research could not indicate a direct impact of probiotics on the energy status of cows, as the implicated indices (NEFA, BHBA and glucose) were not assessed. However, the relationships discussed above presented in the cited publications may partially explain a possible mechanism of probiotic action demonstrated in our study. It can be presumed that the probiotics applied in the experiment were capable of improving the energy balance of cows and also indirectly the activity and burst capacity of phagocytic cells. In addition, probiotics considerably improved the digestibility of the feed consumed by cows and raised the systemic concentrations of energetic components, proteins, vitamins and mineral substances (14, 42). All these substances may be important for the function of immune cells. According to Maldonaldo-Galdeano *et al.* (18), probiotics may stimulate the animal immune system through different mechanisms. Changes in the bacterial flora composition in the gastrointestinal tract may, according to these researchers, redirect the immune response from Th2 (humoral) to Th1 (cellular), maintaining immune homeostasis in the intestines. This means that probiotics can influence immune cells, primarily dendritic cells, T lymphocytes and plasma cells, by stimulating their appropriate subpopulations to produce cytokines and thus become capable of modulating the immune response (5, 13, 28). The direction of the response depends on the kind of probiotic applied, as different probiotic strains may stimulate the secretion of distinct cytokines (7, 34, 37). The mechanism that maintains immune homeostasis is not fully understood. However, it is believed that through stimulation of cytokine secretion, probiotics stimulate the adaptive immune response, in which the predominant role is played by T-regulatory lymphocytes ($CD4^+CD25^+$ and $Foxp3^+$). These cells are capable of producing interleukin 10 (an anti-inflammatory cytokine), through which they modulate the immune response (16, 35). Furthermore, probiotics increase immunoglobulin A (IgA) secretion in the intestines and the neighbouring lymphatic organs, expanding the IgA pool in the peripheral blood and thus protecting other mucous membranes of the organism (3, 6). It is not possible to identify the mechanism stimulating the phagocytic and bactericidal activity of immunocompetent cells in a cow based on the present study results. However, it cannot be excluded that the immunomodulatory effects of probiotics noted in the literature cited also occurred in our research. Similar research conducted by Maldonaldo-Galdeano *et al.* (19) demonstrated increased phagocytic and killing activity of macrophages isolated from the peritoneum and spleen and improvement of immune parameters in mice after application of probiotics as a nutritional supplement. These studies also did not indicate any specific mechanism of the immunomodulatory activity exerted by probiotics.

Conclusion

The present study demonstrated increased phagocytic activity and intracellular killing capacity of phagocytic cells in the peripheral blood of cows following the application of probiotics as a nutritional supplement. It also showed that probiotic application was valid in different lactation periods, as clearly increased immunological indices were obtained in cows that were administered probiotics throughout the duration of the experiment. The results suggest that it can be expected that the maintenance of dairy cows' full immune status with the use of probiotics may facilitate adaptation of their immune system, particularly in the postpartum period, and reduce the incidence of infectious diseases.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

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Animal Rights Statement: The authors declare that the experiments on animals were conducted in accordance with local ethics committee laws and regulations and those of Directive 2010/63/EU. The study was approved by the Local Ethics Committee at the University of Life Sciences in Lublin (approval no. 41/2014). The cows were subjected to the usual veterinary medical procedures, to which the animals' owners agreed.

References

1. Aleri J.W., Hine B.C., Pyman M.F., Mansell P.D., Wales W.J., Mallard B., Fisher A.D.: Periparturient immunosuppression and strategies to improve dairy cow health during the periparturient period. Res Vet Sci 2016, 108, 8–17, doi: 10.1016/j.rvsc.2016.07.007.
2. Bomba A., Nemcova R., Mudronova D., Guba P.: The possibilities of potentiating the efficacy of probiotics. Trends Food Sci Tech 2002, 13, 121–126, doi: 10.1016/S0924-2244(02)00129-2.
3. De Moreno de LeBlanc A., Maldonado-Galdeano C., Chaves S., Perdigón G.: Oral administration of *L. casei* CRL 431 increases immunity in bronchus and mammary glands. Eur J Inflamm 2005, 3, 23–28, doi: 10.1177/1721727X0500300105.
4. Deng Q., Odhiambo J.F., Farooq U., Lam T., Dunn S.M., Ametaj B.N.: Intravaginal lactic acid bacteria modulated local and systemic immune responses and lowered the incidence of uterine infections in periparturient dairy cows. PLoS One 2015, 10, e0124167, doi: 10.1371/journal.pone.0124167.
5. Dogi C.A., Maldonaldo-Galdeano C., Perdigon G.: Gut immune stimulation by non-pathogenic Gram(+) and Gram(–) bacteria. Comparison with a probiotic strain. Cytokine 2008, 41, 223–231, doi: 10.1016/j.cyto.2007.11.014.
6. Fernandez M.F., Boris S., Barbes C.: Probiotic properties of human lactobacilli strains to be used in the gastrointestinal tract. J Appl Microbiol 2003, 94, 449–455, doi: 10.1046/j.1365-2672.2003.01850.x.
7. Foligné B., Parayre S., Cheddani R., Famelart M.H., Madec M.N., Plé C., Breton J., Dewulf J., Jan G., Deutsch S.M.: Immunomodulation properties of multi-species fermented milks. Food Microbiol 2016, 53, 60–69, doi: 10.1016/j.fm.2015.04.002.
8. Gibson G.R., Probert H.M., Van Loo J., Rastall R.A., Robertfroid M.B.: Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. Nutr Res Rev 2004, 17, 259–275, doi: 10.1079/NRR200479.
9. Goff J.P.: Major advances in our understanding of nutritional influences on bovine health. J Dairy Sci 2006, 89, 1292–1301, doi: 10.3168/jds.S0022-0302(06)72197-X.
10. Graugnard D.E., Bionaz M., Trevisi E., Moyes K.M., Salak-Johnson J.L., Wallace R.L., Drackley J.K., Bertoni G., Loor J.J.: Blood immunometabolic indices and polymorphonuclear neutrophil function in peripartum dairy cows are altered by level of dietary energy prepartum. J Dairy Sci 2012, 95, 1749–1758, doi: 10.3168/jds.2011-4579.
11. Ingvartsen K.L., Andersen J.B.: Integration of metabolism and intake regulation: a review focusing on periparturient animals. J Dairy Sci 2000, 83, 1573–1597, doi: 10.3168/jds.S0022-0302(00)75029-6.
12. Ingvartsen K.L., Moyes K.M.: Factors contributing to immunosuppression in the dairy cow during the periparturient period. Jpn J Vet Res 2015, 63, 15–24.
13. Jiang K., Cao S., Cui J.Z., Matsubara J.A.: Immuno-modulatory effect of IFN-gamma in AMD and its role as a possible target for therapy. J Clin Exp Ophthalmol 2013, 2, 71–76, doi: 10.4172/2155-9570-S2-007.
14. Keller D., Farmer S., McCartney A., Gibson G.: *Bacillus coagulans* as a probiotic. Food Sci Tech Bull Funct Foods 2010, 7, 103–109, doi: 10.1616/1476-2137.16015.
15. Kimura K., Reinhardt T.A., Goff J.P.: Parturition and hypocalcemia blunts calcium signals in immune cells of dairy cattle. J Dairy Sci 2006, 89, 2588–2595, doi: 10.3168/jds.S0022-0302(06)72335-9.
16. Lemme-Dumit J.M., Polti M.A., Perdigón G., Maldonaldo-Galdeano C.: Probiotic bacteria cell walls stimulate the activity of the intestinal epithelial cells and macrophage functionality. Benef Microbes 2018, 9, 153–164, doi: 10.3920/BM2016.0220.
17. Luan S., Duersteler M., Galbraith E.A., Cardoso F.C.: Effects of direct-fed *Bacillus pumilus* 8G-134 on feed intake, milk yield, milk composition, feed conversion, and health condition of pre- and postpartum Holstein cows. J Dairy Sci 2015, 98, 6423–6432, doi: 10.3168/jds.2015-9512.
18. Maldonado-Galdeano C., Cazorla S.I., Lemme-Dumit J.M., Vélez E., Perdigón G.: Beneficial effects of probiotic consumption on the immune system. Ann Nutr Metab 2019, 74, 115–124, doi: 10.1159/000496426.
19. Maldonado-Galdeano C., Novotny-Núñez I., de Moreno de LeBlanc A., Carmuega E., Weill R., Perdigón G.: Impact of a probiotic fermented milk in the gut ecosystem and in the systemic immunity using a non-severe protein-energy-malnutrition model in mice. BMC Gastroenterology 2011, 11, 64, doi: 10.1186/1471-230X-11-64.
20. Martinez N., Sinedino L.D.P., Bisinotto R.S., Ribeiro E.S., Gomes G.C., Lima F.S., Greco L.F., Risco C.A., Galvão K.N., Taylor-Rodriguez D., Driver J.P., Thatcher W.W., Santos J.E.P.: Effect of induced subclinical hypocalcemia on physiological responses and neutrophil function in dairy cows. J Dairy Sci 2014, 97, 874–887, doi: 10.3168/jds.2013-7408.
21. McArt J.A.A., Nydam D.V., Oetzel G.R.: Epidemiology of subclinical ketosis in early lactation dairy cattle. J Dairy Sci 2012, 95, 5056–5066, doi: 10.3168/jds.2012-5443.
22. Mulligan F.J., Doherty M.L.: Production diseases of the transition cow. Vet J 2008, 176, 3–9, doi: 10.1016/j.tvjl.2007.12.018.
23. Nocek J.E., Kautz W.P.: Direct-fed microbial supplementation on ruminal digestion, health, and performance of pre- and postpartum dairy cattle. J Dairy Sci 2006, 89, 260–266, doi: 10.3168/jds.S0022-0302(06)72090-2.
24. Nocek J.E., Kautz W.P., Leedle J.A.Z., Block E.: Direct-fed microbial supplementation on the performance of dairy cattle during the transition period. J Dairy Sci 2003, 86, 331–335, doi: 10.3168/jds.S0022-0302(03)73610-8.
25. Oelschlaeger T.A.: Mechanisms of probiotic actions – a review. Int J Med Microbiol 2010, 300, 57–62, doi: 10.1016/j.ijmm.2009.08.005.
26. Panasiuk A., Wysocka J., Maciorkowska E., Panasiuk B., Prokopowicz D., Zak J., Radomski K.: Phagocytic and oxidative burst activity of neutrophils in the end stage of liver cirrhosis. World J Gastroenterol 2005, 11, 7661–7665, doi: 10.3748/wjg.v11.i48.7661.
27. Peng H., Wang J.Q., Kang H.Y., Dong S.H., Sun P., Bu D.P., Zhou L.Y.: Effect of feeding *Bacillus subtilis natto* fermentation product on milk production and composition, blood metabolites and rumen fermentation in early lactation dairy cows. J Anim Physiol Anim Nutr 2012, 96, 506–512, doi: 10.1111/j.1439-0396.2011.01173.x.
28. Petrof E.O., Kojima K., Ropeleski M.J., Musch M.W., Tao Y., De Simone C., Chang E.B.: Probiotics inhibit nuclear factor-kappaB and induce heat shock proteins in colonic epithelial cells through proteasome inhibition. Gastroenterology 2004, 127, 1474–1487, doi: 10.1053/j.gastro.2004.09.001.
29. Punetha M., Roy A.K., Ajithakumar H.M., Para I.A., Gupta D., Singh M., Bharati J.: Immunomodulatory effects of probiotics and prilled fat supplementation on immune genes expression and lymphocyte proliferation of transition stage Karan Fries cows. Vet World 2018, 11, 209–214, doi: 10.14202/vetworld.2018.209-214.
30. Ribeiro E.S., Lima F.S., Greco L.F., Bisinotto R.S., Monteiro A.P.A., Favoreto M., Ayres H., Marsola R.S., Martinez N., Thatcher W.W., Santos J.E.P.: Prevalence of periparturient diseases and effects on fertility of seasonally calving grazing dairy

cows supplemented with concentrates. J Dairy Sci 2013, 96, 5682–5697, doi: 10.3168/jds.2012-6335.
31. Saulnier D.M.A., Spinler J.K., Gibson G.R., Versalovic J.: Mechanisms of probiosis and prebiosis: considerations for enhanced functional foods. Curr Opin Biotechnol 2009, 20, 135–141, doi: 10.1016/j.copbio.2009.01.002.
32. Scalia D., Lacetera N., Bernabucci U., Demeyere K., Duchateau L., Burvenich C.: In vitro effects of nonesterified fatty acids on bovine neutrophils oxidative burst and viability. J Dairy Sci 2006, 89, 147–154, doi: 10.3168/jds.S0022-0302(06)72078-1.
33. Schrezenmeir J., de Vrese M.: Probiotics, prebiotics and synbiotics—approaching a definition. Am J Clin Nutr 2001, 73, 361–364, doi: 10.1093/ajcn/73.2.361s.
34. Sheikhi A., Giti H., Heibor M.R., Jafarzadeh A., Shakerian M., Baharifar N., Niruzad F., Moghaddam A.S., Kokhaei P., Baghaeifar M.: *Lactobacilus delbrueckii* subsp. *bulgaricus* modulates the secretion of Th1/Th2 and Treg cell-related cytokines by PBMCs from patients with atopic dermatitis. Drug Res 2017, 67, 724–729, doi: 10.1055/s-0043-117612.
35. Sichetti M., De Marco S., Pagiotti R., Traina G., Pietrella D.: Anti-inflammatory effect of multistrain probiotic formulation (*L. rhamnosus*, *B. lactis*, and *B. longum*). Nutrition 2018, 53, 95–102, doi: 10.1016/j.nut.2018.02.005.
36. Sordillo L.M., Mavangira V.: The nexus between nutrient metabolism, oxidative stress and inflammation in transition cows. Anim Prod Sci 2014, 54, 1204–1214, doi: 10.1071/AN14503.
37. Takeda S., Kawahara S., Hidaka M., Yoshida H., Watanabe W., Takeshita M., Kikuchi Y., Bumbein D., Muguruma M., Kurokawa M.: Effects of oral administration of probiotics from Mongolian dairy products on the Th1 immune response in mice. Biosci Biotechnol Biochem 2013, 77, 1372–1378, doi: 10.1271/bbb.120624.
38. Uyeno Y., Shigemori S., Shimosato T.: Effect of Probiotics/Prebiotics on Cattle Health and Productivity. Microbes Environ 2015, 30, 126–132, doi: 10.1264/jsme2.ME14176.
39. van Knegsel A.T.M., de Vries Reilingh G., Meulenberg S., van den Brand H., Dijkstra J., Kemp B., Parmentier H.K.: Natural antibodies related to energy balance in early lactation dairy cows. J Dairy Sci 2007, 90, 5490–5498, doi: 10.3168/jds.2007-0289.
40. Wan M.L.Y., Forsythe S.J., El-Nezami H.: Probiotics interaction with foodborne pathogens: a potential alternative to antibiotics and future challenges. Crit Rev Food Sci Nutr 2019, 59, 3320–3333, doi: 10.1080/10408398.2018.1490885.
41. Wankhade P.R., Manimaran A., Kumaresan A., Jeyakumar S., Ramesha K.P., Sejian V., Rajendran D., Varghese M.R.: Metabolic and immunological changes in transition dairy cows: A review. Vet World 2017, 10, 1367–1377, doi: 10.14202/vetworld.2017.1367-1377.
42. Yoo J.Y., Kim S.S.: Probiotics and prebiotics: present status and future perspective on metabolic disorders. Nutrients 2016, 8, 173, doi: 10.3390/nu8030173.