Research in Veterinary Science 96 (2014) 365–370

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Intravaginal administration of lactic acid bacteria modulated the incidence of purulent vaginal discharges, plasma haptoglobin concentrations, and milk production in dairy cows

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ARTICLE INFO

Article history:
Received 6 August 2013
Accepted 9 February 2014

Keywords:
Lactic acid bacteria
Metritis
Reproduction
Haptoglobin
Dairy cow

ABSTRACT

This investigation studied the effects of intravaginal administration of a mixture of lactic acid bacteria (LAB) on the incidence of purulent vaginal discharges (PVD), plasma haptoglobin concentrations, and milk production in dairy cows. A total of 82 pregnant primiparous and multiparous Holstein dairy cows were used in this study. Half of the cows received intravaginally 1 mL of LAB at 10^{10}–10^{12} cfu/mL and the other half 1 mL of reconstituted skim milk (i.e., carrier) (controls). Administration of LAB was conducted once per wk during 2 and 1 wk before the expected day of calving and at 1, 2, 3, and 4 wk postpartum. Data demonstrated that intravaginal administration of LAB decreased the occurrence of PVD at 3 wk postpartum ($P < 0.05$). Concentrations of plasma haptoglobin, an acute phase protein often associated with uterine infections, was lower in cows treated with the LAB mixture at 2 wk ($P < 0.001$) and 3 wk ($P < 0.05$) postpartum. Treatment with LAB did not improve overall pregnancy rate, but the treated multiparous cows produced more milk than their control counterparts ($P < 0.05$), whereas no difference was observed in primiparous cows regarding milk yield ($P > 0.05$). Overall, this is the first study demonstrating that intravaginal LAB administration lowers the incidence of PVD and enhances milk production in dairy cows. Further research is warranted to evaluate the effects of LAB on reproductive performance in a larger cohort of cows.

1. Introduction

Uterine infections affect early postpartum dairy cows lowering their reproductive and productive performance. Moreover, cows affected by uterine infections are at a higher culling risk due to infertility, which has negative impacts on the sustainability of dairy industry. Recent reports indicate that the number one reason for culling of dairy cows is poor reproductive performance. Indeed, data from Canada indicate that almost 30% of all dairy cows culled for sickness were for infertility reasons (CanWest DHI Canada, 2010).

Under normal conditions the vaginal tract of a dairy cow is populated by a diversity of bacteria dominated mainly by lactic acid bacteria (LAB) (Otero et al., 2006; Rodriguez et al., 2011; Wang et al., 2013). Recently we showed that bacilli and LAB of the genera *Enterococcus*, *Lactobacillus*, and *Pediococcus* were present in the vaginal tracts of both healthy and infected cows (Wang et al., 2013). However, the infected cows a tremendous increase in the vaginal bacteria population that consisted mainly of *Escherichia coli*. Moreover, three *E. coli* isolates harbored the gene coding for Shiga-like-toxin (SLT) I or II (Wang et al., 2013).

An increasing body of evidence indicates that *Lactobacillus* strains suppress the growth of other endogenous bacteria in the vagina through the production of organic acids such as lactic acid, H_2O_2, and bacteriocins (Aroutcheva et al., 2001). The production of organic acids maintains the vaginal pH at acidic values, thereby creating an inhospitable environment for the growth of most endogenous pathogenic bacteria (Reid, 2002).

Although several investigators have suggested utilization of LAB, isolated from the reproductive tract of healthy cows, as prophylactic or treatment interventions against uterine infections in dairy cattle (Kummer et al., 1997; Otero et al., 2006; Nader-Macías et al., 2008; Rodriguez et al., 2011), to our best knowledge, there have been no

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http://dx.doi.org/10.1016/j.rvsc.2014.02.007
0034-5288/

reports of controlled trials in dairy cows. We hypothesized that application of LAB in the vagina of transition dairy cows several times around parturition might improve health and enhance the overall reproductive and productive performance in dairy cows. Therefore this study was designed to test the effects of intravaginal administration of a mixture of one isolate of *Lactobacillus sakei* and two isolates of *Pediococcus acidilactici*, starting at 2 wk before the expected day of parturition until 4 wk after parturition, on plasma haptoglobin, a protein produced by the liver as part of the acute phase response, often associated with metritis (Huzzey et al., 2009), incidence of vaginal discharges, and reproductive and productive performance of transition dairy cows.

2. Materials and methods

2.1. Animals, preparation of probiotic bacteria, and treatments

The experiment was conducted at the Dairy Research and Technology Centre, University of Alberta. A total of 82 (30 primiparous and 52 multiparous) Holstein dairy cows (overall parity of the calving event that occurred during the study was 2.4 ± 1.5; mean ± SD) with an average body condition score (BCS) of 3.5 (Edmonson et al., 1989) were assigned to the present study. All animals were cared for according to the guidelines established by the Canadian Council on Animal Care, and the experimental protocol was approved by the University of Alberta Animal Care and Use Committee for Livestock. Cows were randomly allocated into 2 different groups (n = 41 cows/group), as control (CTR) and LAB group in a randomized block design. Cows were blocked by the expected day of calving, parity, BCS at 2 wk before the expected day of calving, and milk production on previous lactation. Restricted block randomization was employed to ensure equal treatment numbers at equally spaced points in the sequence of assignments of the experimental units (i.e., cows). For this, PROC PLAN of SAS (version 9.1.2, SAS Institute Inc., NC, USA) was used for random number generation (random permutations). Blocks were built to equally consider different important parameters such as parity, milk yield, and susceptibility to disease in previous lactations as well as BCS. To limit the occurrence of potential bias in conducting, analyzing, and interpreting the trial data, the personnel involved was blinded to treatments during data collection, clinical observations as well as lab analyses and statistical analysis of the data. A power test analysis was also conducted to determine the approximate sample size of this experiment. Cows of the LAB group were administered intravaginally, using a sterile plastic insemination pipette, once per wk on wk −2 and −1 before the expected day of calving and on wk 1, 2, 3, and 4 after calving with 10^{10}–10^{12} cfu of LAB/wk/cow.

The LAB cultures were prepared by separately growing each strain of *L. sakei* FUA 3089, *P. acidilactici* FUA 3140, and *P. acidilactici* FUA 3138 in 250 ml of mMRS broth overnight. Bacterial cells were collected by centrifuging at 5525×*g* for 20 min using the Allegra 25R Centrifuge (Beckman Coulter, Mississauga, Canada). The pellets of all three strains were resuspended and combined in 150 ml of 10% skim milk. Aliquots of 250 μL of the probiotic mixture were made and these samples were freeze-dried at −70 °C using the Freeze Dry System/Freezone 4.5 (Labconco, Kansas City, USA). Prior to treatment, the freeze-dried probiotic mixture was reconstituted in 1 mL sterile 0.9% saline. The control treatment was prepared by making 1 mL aliquots with autoclaved 10% skimmed milk and the samples were stored at −20 °C. Both the probiotic and control treatments were administered intravaginally into the cows within 2 h of being taken out of storage at −20 °C. Survival of the cultures during strain preparation and storage was monitored by determination of cell counts. Relative to the initial cell count of the overnight cultures, the survival was 73% after freezing and 38% after freeze-drying. No decrease in cell counts was observed during frozen storage of the freeze-dried cells. The three strains were found to be compatible as a mixture and all three strains were recovered from the freeze-dried preparation. Enumeration results showed that at least 10^{10} cells/mL were used for treatments.

Cows of the CTR group received an intravaginal carrier (i.e., 1 mL of autoclaved skimmed milk) once per wk, for 6 wk. A dried mixture of 3 probiotic bacteria (*L. sakei* FUA 3089, *P. acidilactici* FUA 3140, and *P. acidilactici* FUA 3138), prepared and stored at −20 °C in 3 mL vials, was reconstituted in 1 mL sterile 0.9% saline and administered intravaginally to the cows within 2 h. Methodologies for identification and growth of bacteria are described elsewhere (Wang et al., 2013). The probiotic load was gently deposited through an aseptic manner into the cranio-medial part of the vagina using a sterile insemination pipette and 5 mL plastic syringe. Before administration vulva was thoroughly washed with warm water and soap, dried with paper towel, and disinfected with iodine solution. All animals were offered a close-up total mixed ration (TMR) starting at 3 wk prepartum, and they were gradually switched to the lactation TMR during the first 7 d after parturition, and fed the same diet until the end of the experiment, as described previously (Zebeli et al., 2011). Diets were formulated and offered to the cows to meet or exceed the energy and nutrient requirements of dry and lactating cows as per the NRC (2001) guidelines. Feed samples and orts were taken periodically and were analyzed to assure the daily intake of all calculated nutrients.

2.2. Clinical observations

All cows were monitored starting at 2 wk before the expected day of calving up to the next successful pregnancy. Clinical records were collected as following: (1) daily monitoring of general appearance (from −2 wk up to +4 wk) and rectal temperature (from −1 wk up to +3 wk) as well as feed intake (from −2 wk up to +8 wk) and milk production during the +8 wk of lactation; (2) veterinary treatments throughout the lactation period; (3) vaginal examinations by a vaginal speculum at wk 3 and 5 postpartum (at 07:00–08:00 h) for presence of abnormal vaginal discharges, including their amount, presence or absence of pus, blood, and other abnormal signs such as foul-smelling odor and abnormal color. The vaginal examinations were classified as purulent vaginal discharge (PVD) or foul-smelling purulent discharge. The PVD was characterized by the presence of purulent discharge (>50% pus) at 3 wk after parturition, and mucopurulent (approximately 50% pus, 50% mucus) discharge detectable in the vagina at 5 wk postpartum (Sheldon et al., 2008). No specific medical treatments were administered to the cows diagnosed with PVD or foul-smelling purulent discharges. Only cows showing general sickness symptoms were treated according to management practices of the dairy farm.

The breeding protocol consisted in synchronization of the ovulation followed by timed AI (Ovsynch/timed AI; Ambrose et al., 2010). This protocol involved a first intramuscular administration of 2 mL of GnRH (Fertiline; Vetoquinol NA, Lavaltrie, Quebec, Canada) day 60 after parturition followed by intramuscular administration of 2 mL of Lutalyse (10 mg dinoprost; Pharmacia & Upjohn Animal Health, Orangeville, ON, Canada) 7 d later, and a 2nd injection of 2 mL Fertiline after 2 d, and cows were artificially inseminated approximately 16 h after this 2nd GnRH treatment, without estrus detection. Cows declared non-pregnant were submitted to the same synchronization protocol described above and were re-inseminated. The diagnosis of pregnancy was confirmed by transrectal ultrasonography twice at 32 and 60 d after insemination. According to the farm management policy, non-pregnant cows were inseminated until a maximum of 5 consecutive times. All breeding records including 1st insemination pregnancy rate