Intravaginal probiotics modulated metabolic status and improved milk production and composition of transition dairy cows


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ABSTRACT: The objective of this investigation was to evaluate whether intravaginal infusion of probiotics (a lactic acid bacteria cocktail) around parturition would influence metabolic status and increase milk production of transition dairy cows. One hundred pregnant Holstein dairy cows were assigned to 1 of the 3 experimental groups receiving intravaginal infusion of probiotics or carrier (i.e., sterile skim milk) once a week at wk –2, –1, and +1 relative to calving as follows: 2 consecutive probiotics before parturition and 1 carrier dose after parturition (TRT1), 3 consecutive probiotics doses around parturition (TRT2), and 3 consecutive carrier doses around parturition (CTR). The probiotics were a lyophilized culture mixture composed of Lactobacillus sakei FUA3089 and Pediococcus acidilactici FUA3138 and FUA3140 with a cell count of 10^8 to 10^9 cfu/dose. Blood was sampled from wk –2 to +3 and milk was sampled on the third day in milk (DIM) and from wk +1 to +5 on a weekly basis. Feed intake and milk production was monitored until wk +8. Results showed that the TRT2 group (366.12 ± 49.77 μmol/L) had a lower (P = 0.01) concentration of NEFA in the serum than the CTR group (550.85 ± 47.16 μmol/L). The concentrations of IgG in the milk were 32.71 ± 3.00 mg/mL in the TRT1 group, 17.47 ± 4.54 mg/mL in the TRT2 group, and 6.73 ± 3.43 mg/mL in the CTR group at 3 DIM (P < 0.01). Meanwhile, both the TRT1 and the TRT2 group had lower haptoglobin in the milk compared with the CTR group at 3 DIM (P < 0.01). The TRT1 group had greater milk protein content than the CTR group (2.99 ± 0.04 vs. 2.82 ± 0.04%; P = 0.02), whereas the TRT2 group tended to have greater lactose content compared with the CTR group (4.53 ± 0.03 vs. 4.44 ± 0.03%; P = 0.05). The effect of treatment interacted with parity with regards to milk production and feed efficiency. Multiparous cows in the TRT1 and TRT2 groups had greater milk production and feed efficiency than those in the CTR group (P < 0.01 and P = 0.02, respectively). Among primiparous cows, those in the TRT2 group had greater milk production (P = 0.04) whereas those in the TRT1 group had lower feed intake (P < 0.01) than those in the CTR group. Both the TRT1 and the TRT2 groups had enhanced feed efficiency compared with the CTR group (P < 0.01). In conclusion, intravaginal infusion of lactic acid bacteria modulated concentrations of selected serum metabolites and milk components and increased milk efficiency of transition dairy cows.

Key words: dairy cow, lactic acid bacteria, metabolite, milk production


INTRODUCTION

Transition dairy cows experience major metabolic alterations due to parturition, commencement of lactation, and dietary changes. There is a large energy flow towards the mammary gland immediately after calving and a commonly observed decrease in feed intake resulting in a negative energy balance, which has been associated with the high incidence of periparturient diseases, such as metritis, retained placenta, ketosis, mastitis, and milk fever (Bewley et al., 2008; Sordillo et al., 2009; Leblanc, 2010; Giuliodori et al., 2013). Postpartum infection of the uterus (i.e., metritis) affects 40% of the cows and bacteremia affects 20 to 30% of the infected cows (Sheldon et al., 2009; Credille et al., 2014). Endotoxins present in lochia could translocate...
into the systemic circulation and affect carbohydrate and lipid metabolism. Several authors have indicated that circulatory endotoxins increase blood lactate and modulate concentrations of glucose (Kun and Miller, 1948; Roy et al., 1976; Spitzer et al., 1989). It has been also reported that endotoxins induce hyperlipidemia (Feingold et al., 1992; Hardardóttir et al., 1995) and increase high-density lipoproteins in the circulation (Hardardóttir et al., 1996). Recently, Zebeli et al. (2013) observed that cows orally administered lipopolysaccharide had lower concentrations of NEFA and β-hydroxybutyric acid (BHBA) and greater insulin and tended to have elevated plasma glucose. Acute metritic cows also display greater concentrations of acute phase proteins in the milk including milk amyloid A (MAA) and haptoglobin (Hp; Nazifi et al., 2008; Khoshvaghti et al., 2009). Furthermore, metritis is associated with lower feed intake and milk production in dairy cows (Wittrock et al., 2011).

Lactic acid bacteria (LAB) are well known for their ability to enhance immunity and have been used for preventing and treating diseases including urogenital infections in humans (Reid and Bruce, 2003; Doron and Gorbach, 2006). In addition to the application to human subjects, LAB have demonstrated characteristics that potentiate their use in preventing uterine infections and mastitis of dairy cattle (Otero et al., 2006; Otero and Nader-Macias, 2006; Nader-Macias et al., 2008; Espeche et al., 2009, 2012). Data from this same investigation also showed a lower incidence rate of uterine infections in cows intravaginally treated with LAB (Deng et al., 2015). Therefore, we hypothesized that treatment of transition dairy cows with 2 or 3 intravaginal doses of LAB around calving would influence metabolic responses and milk production and composition. Accordingly, the objectives of this study were to evaluate if intravaginal infusion of 2 to 3 doses of LAB around parturition would affect the metabolic status, milk production, and composition in transition dairy cows.

**MATERIALS AND METHODS**

**Animals and Experimental Design**

All experimental procedures were approved by the University of Alberta Animal Care and Use Committee for Livestock (animal use protocol number 120). One hundred pregnant Holstein cows (78 multiparous and 22 primiparous) were randomly assigned to 1 of 3 treatment groups by sequential stratified block design according to parity (i.e., primiparous or multiparous), BCS at wk –3 (thin, <3/5, or not thin ≥3/5), and milk production of the previous lactation (305 d ≥9,000 or <9,000 kg). Cows received intravaginal probiotics or carrier (sterile skim milk) once a week at wk –2, –1, and +1 relative to calving as follows: 2 consecutive probiotics before parturition and 1 carrier dose after parturition (TRT1; n₁ = 34), 3 consecutive probiotics doses around parturition (TRT2; n₂ = 32), and 3 consecutive carrier doses around parturition (CTR; n₃ = 34). Probiotics used in this study were a lyophilized culture mixture of 3 LAB composed of Lactobacillus sakei FUA3089 and Pediococcus acidilactici FUA3138 and FUA3140, which was infused at 10⁸ to 10⁹ cfu/dose. These bacteria were isolated, identified, and cultured from the vaginal mucus of prepartal Holstein dairy cows at the same dairy farm where this study was conducted. The LAB cocktail was prepared and provided by the Microbiology Laboratory in the Department of Agricultural, Food and Nutritional Science at University of Alberta (Edmonton, AB, Canada). More details can be found in an article published by Wang et al. (2013) in our team.

Both LAB and carrier were stored at –86°C in vials in the form of dry powder, and each vial was reconstituted in 1 mL sterile 0.9% saline before administration. After blood sampling, cows were stimulated to urinate by massaging the perineal area. Then, the external genital area of cows was washed with warm water and soap and dried with paper towels followed by a 30% iodine spray before treatment with LAB. The LAB or carrier was gently infused into the vaginal tract with individually wrapped sterile drilled infusion tubes (Continental Plastic Corp., Delavan, WI) capped with a 5-mL screw-tip sterile syringe (Becton, Dickinson and Company, Franklin Lakes, NJ) and deposited at the cranial vagina. Aseptic procedures were maintained during LAB or carrier administration. Cows in the same period were fed with the same diet. All cows were housed in individual tie stalls with free access to water, fed with a total mixed ration (Table 1) once daily at 0800 h (and rations were fed ad libitum to allow for approximate 10% refusal based on the feed intake on the previous day), and milked twice a day at 0500 and 1600 h.

**Sampling and Clinical Monitoring**

Blood samples were collected from the coccygeal vein once a week before the morning feeding (0800 h) with 10-mL vacutainer tubes without anticoagulant (BD Vacutainer Systems, Plymouth, UK) from wk 2 before the expected day of parturition to wk 3 after parturition. Blood samples were kept on ice after collection, until coagulation (30 min), and then centrifuged at 2,090 × g at 4°C for 20 min (Beckman Coulter, Pasadena, CA) to separate serum. Serum samples were stored in sterile plastic tubes at –20°C until analyses.

Milk samples were collected on the third day in milk (DIM) as well as from wk 1 to 5 postpartum on a weekly basis. For each milk sample, 2 subsamples from the same
cow were collected at 0500 and 1600 h, respectively, and then mixed half and half to form 1 sample. Daily milk production was monitored from wk 1 to 8 postpartum. Daily feed intake was calculated as the difference between feed given and orts from wk –2 to +8.

Body condition score was evaluated at wk –3, –1, +3, +6, and +9 on a 5-point scale at an interval of 0.25, referring to the judgment criteria provided by University of Arkansas (Kellogg, 2015). The evaluation was conducted by the same technician, who was blinded to the treatment groups.

**Laboratory Analyses**

Based on the data from a pilot study previously conducted by our group (Ametaj et al., 2014), 100 dairy cows in total were enrolled in this study in order to acquire a power of 85% to detect treatment differences in uterine infections and 10 samples from each group to detect treatment differences in blood variables, acute phase proteins, and IgG in the milk. Considering the incidence rate of mastitis is approximately 50%, 20 milk samples for each group were used for the composition assay. Parity did not influence blood variables and milk components in this study and did not improve the model and, therefore, was excluded from the final model.

The incidence rates of metritis were 15% in the TRT1 group, 6% in the TRT2 group, and 38% in the CTR group (Deng et al., 2015). Although there were 12 metritic cows out of 32 in the CTR group, there were only 5 and 2 in the TRT1 and TRT2 groups, respectively. A statistical result from 2 replicates is not reliable. In order to make comparable conclusions, for all the variables, all the samples used were from clinically healthy cows. A subset of serum samples from the first 10 healthy cows in each group were used to evaluate concentrations of glucose, insulin, NEFA, BHBA, cholesterol, and lactate. A subset of milk samples from the first 30 cows (10 per group) collected on 3, 14, and 35 DIM were used to determine the concentrations of MAA, Hp, and total IgG. All samples were tested in duplicate. Both the inter- and intra-assay CV were less than 10% for all the variables. A subset of milk samples from the first 60 cows (20 per group) collected on +1 to +5 wk were analyzed at Central Milk Testing Laboratory (Edmonton, AB, Canada) for milk fat, protein, lactose, milk urea nitrogen, total solid contents, and somatic cell count using mid-infrared spectroscopy (MilkoScan 665; N. Foss Electric A/S, Hillerød, Denmark).

Concentrations of glucose in serum were quantified by an enzymatic method with a commercial kit (Genzyme Diagnostics P. E. I. Inc., Charlottetown, PE, Canada). Briefly, glucose in samples is first phosphorylated into glucose-6-phosphate. Then, the oxidation of glucose-6-phosphate leads to production of NADH, which produces a color proportional to the glucose concentration in the sample. The serum glucose was then determined by reading on a microplate spectrophotometer (Spectramax 190; Molecular Devices Corp., Sunnyvale, CA) at 340 nm. According to the manufacturer’s instructions, the lower limit of detection of the test was 0.06 mg/dL.

Concentration of insulin in the serum was measured using a bovine insulin ELISA kit (Mercodia AB, Uppsala, Sweden) based on a sandwich technique. The insulin in the samples is bound by both the anti-insulin monoclonal antibody coated on the plate and peroxidase-conjugated anti-insulin monoclonal antibody. The enzyme catalyzes a 3,3’,5,5’-tetramethylbenzidine reaction, giving a blue color, which is converted into yellow when acid is added to stop the reaction and read spectrophotometrically at 450 nm.

Concentrations of NEFA in the serum were determined by an enzymatic colorimetric method using a commercially available kit (Randox Laboratories Limited, Crumlin, UK). Coenzyme A is acylated by fatty acids in the sample in presence of acyl-CoA synthetase and then produces hydrogen peroxide in presence

### Table 1. Ingredients and chemical composition of the diets for cows

<table>
<thead>
<tr>
<th>Item</th>
<th>Prepartum diet</th>
<th>Early lactation diet</th>
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<tr>
<td>Ingredient, % of DM</td>
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<tr>
<td>Alfalfa hay</td>
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<tr>
<td>Barley silage</td>
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<tr>
<td>CUD grain</td>
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<td>–</td>
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<td>Dairy supplement</td>
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**Chemical composition**

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<th>Item</th>
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<th>NE lactation, Mcal/kg</th>
<th>TDN, % of DM</th>
<th>NDF, % of DM</th>
<th>ADF, % of DM</th>
<th>CP, % of DM</th>
<th>Calcium, % of DM</th>
<th>Phosphorus, % of DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, %</td>
<td>49.45</td>
<td>57.98</td>
<td>68.95</td>
<td>44.99</td>
<td>23.73</td>
<td>15.45</td>
<td>0.96</td>
<td>0.43</td>
</tr>
</tbody>
</table>

1. CUD = – closed-up diet; contains: 55.0% rolled barley grain, 7.5% canola meal, 6.2% Dairy dry cow micro-premix (Land O’Lakes, Inc., Fort Dodge, IA), 8.7% limestone, 15.7% Animate (Phibro Animal Health Corporation, Teaneck, NJ), 0.9% molasses, 4.1% canola oil, and 1.7% yeast.

2. Dairy supplement contains: 0.056% ADE Vit (UFA Limited, Calgary, Canada), 0.10% Ruminant TM Pak (Wetaskiwin Co-operative Association Ltd., Wetaskiwin, Canada), 0.07% Selenium, 0.06% Custom TM Complex (Wetaskiwin Co-operative Association Ltd., Wetaskiwin, Canada), 1.25% Di-calcium phosphate, 10% corn distillers grain (Co-op Atlantic, Truro, NS), 25% corn ground, 30% corn rolled, 0.015% vitamin D (10,000 kIU/kg); 0.14% Diamond V XPC (Diamond V Mills, Cedar Rapids, IA), 1.00% Enteria (Archer Daniels Midland Company, Chicago, IL), 2.00% Fermenten (Church & Dwight Co. Inc., Ewing, NJ), 1.5% limestone, 0.37% Mag Oxmagnesium oxide -50%, 15.5% canola meal, 2.75% Amino Plus (Lignotech USA, Inc., Rothschild, WI), 6.5% soy bean meal (47.5%), 1% sodium bicarbonate, 0.11% salt, 2.45% cattle tallow, 0.007% biotin 2%, and 0.015% ADM Vit E 405 (Archer Daniels Midland Company, Chicago, IL).
of acyl-CoA oxidase. Hydrogen peroxide is oxidized by peroxidase in the presence of 3-methyl-N-ethyl-N(β-hydroxyethyl) aniline with 4-aminodipyrine to form a purple adduct, which is proportional to the concentration of NEFA in the sample. The optical density was measured at 550 nm on a microplate spectrophotometer (Spectramax 190; Molecular Devices Corp.). The lower limit of detection of the assay was 0.50 mmol/L.

Quantitation of serum BHBA was done using a commercially available kit (Stanbio Laboratory, Boerne, TX). Briefly, BHBA is converted to acetooacetate and NADH at pH 8.5 by β-hydroxybutyrate dehydrogenase in presence of NAD\(^+\), resulting in the production of NADH, which reacts with 2-p-iodophenyl-3-p-nitrophenyl-5-phenyltetrazolium chloride (INT) in presence of iophorase and produces a color proportional to the concentration of BHBA in the sample. The plate was finally read at 505 nm on a microplate spectrophotometer (Spectramax 190; Molecular Devices Corp.). The lower limit of detection of the assay was 0.125 µmol/L.

The concentration of serum cholesterol was determined by using an enzymatic colorimetric method with a commercial kit (Genzyme Diagnostics P.E.I. Inc.). The cholesterol esters are hydrolyzed to free cholesterol by cholesterol esterase. The free cholesterol is then oxidized by cholesterol oxidase to cholesten-3-one with the simultaneous production of hydrogen peroxide. The hydrogen peroxide produced couples with 4-aminodipyrine and phenol, in the presence of peroxidase, to yield a chromogen with maximum absorbance at 505 nm.

Concentration of serum lactate was measured using a kit (Biomedical Research Service Center of University at Buffalo, Buffalo, NY) based on the reduction of the tetrazolium salt INT in a NADH-coupled enzymatic reaction to formazan, which is water soluble and exhibits an absorption maximum at 492 nm. The intensity of the color is in proportional to the lactate concentration.

Milk amyloid A was measured using the Tridelta Mast ID range MAA kit (Tridelta Development Limited, Maynooth, Co., Kildare, Ireland). Milk samples were diluted 50-fold prior to testing. The intensity of the color produced is proportional to the concentration of MAA present in the sample. The sensitivity of MAA assay was 0.10 µg/mL. Milk Hp was measured using a bovine Hp ELISA test kit (Life Diagnostics Inc., West Chester, PA). Milk samples were diluted 20-fold prior to testing. The minimum concentration of Hp standard was 7.8 ng/mL. Concentrations of total IgG in the milk were measured with bovine IgG ELISA kits (Alpha Diagnostic Intl. Inc., San Antonio, TX). Milk samples were originally diluted 1:100,000 by 3 dilutions prior to testing. For those samples whose concentration was still out of the standard range, samples were diluted more until they fell within the range. The minimum concentration of standard was 10 mg/mL. The principle of all these 3 assays is a solid phase sandwich ELISA similar to that of insulin.

Statistical Analyses

Feed efficiency was calculated as the ratio of milk production to feed intake from +1 to +8 wk. All data, including blood variables, milk components, milk production, feed intake, feed efficiency, and BCS, were analyzed using SAS 9.2 software (SAS Inst. Inc., Cary, NC) with a repeated design structure in a MIXED model incorporating treatment, week, and parity. The covariance structure was modeled using first order autoregressive for the repeated measurements over time. A post hoc test was conducted if the interaction between the treatment and days was significant. Significance was declared at $P < 0.05$ and tendency at $0.05 \leq P < 0.10$.

RESULTS

Effect of Lactic Acid Bacteria Treatment on Metabolic Status

Based on the data from a pilot study previously conducted by our group (Ametaj et al., 2014), 100 dairy cows in total were enrolled in this study in order to acquire a power of 85% to detect treatment differences in uterine infections and 10 samples from each group to detect treatment differences in blood variables, acute phase proteins, and IgG in the milk. Considering the incidence rate of mastitis is approximately 50%, 20 milk samples for each group were used for the composition assay. Parity did not influence blood variables and milk components in this study, and neither improved the model, and, therefore, was excluded from the final model.

Concentrations of NEFA in the serum differed among treatment groups ($P < 0.01$; Fig. 1). Cows in the TRT2 group (366.12 ± 49.77 µmol/L) had lower concentrations of NEFA in the serum than both TRT1 (633.16 ± 57.61 µmol/L; $P < 0.01$) and CTR cows (550.85 ± 47.16 µmol/L; $P = 0.01$). There was no difference between TRT1 and CTR cows with regards to serum NEFA. Serum NEFA also varied over time ($P < 0.01$). There was no significant interaction between treatment and week.

Intravaginal LAB treatment exerted a significant effect on the concentration of cholesterol in the serum of dairy cows ($P < 0.01$; Fig. 2). Cows in the TRT1 (107.82 ± 3.85 vs. 95.04 ± 3.82 mg/dL; $P = 0.03$) and TRT2 (115.69 ± 3.82 vs. 95.04 ± 3.82 mg/dL; $P < 0.01$) groups both had greater cholesterol than cows in the CTR group. All cows had increased concentration of cholesterol after calving compared with precalving levels ($P < 0.01$). No significant interactions were observed between treatment and week.
Lactic acid bacteria treatment increased concentration of lactate in the serum ($P < 0.01$; Fig. 3). Cows in both the TRT1 ($P < 0.01$) and the TRT2 ($P = 0.02$) groups had greater concentrations of lactate than those in the CTR group (1.32 ± 0.08, 1.21 ± 0.07, and 0.97 ± 0.07 mmol/L in TRT1, TRT2, and CTR, respectively). No difference was observed between the TRT1 and TRT2 groups. There was no effect of week or interaction between treatment and week on the concentration of lactate.

Concentrations of BHBA in the serum were not affected by LAB treatment ($P = 0.29$) but varied over time ($P < 0.01$; Fig. 4). There was no interaction between treatment and week. Concentrations of glucose and insulin in the serum were not affected by treatment, week, or their interaction (data not presented).

**Effect of Lactic Acid Bacteria Treatment on Immunoglobulin and Acute Phase Proteins in Milk**

There was an effect of treatment by DIM regarding the concentration of total IgG in the milk ($P = 0.01$; Fig. 5). At 3 DIM, cows in the TRT1 group had a greater concentration of IgG than those in the CTR group (32.71 ± 3.00 vs. 6.73 ± 3.43 mg/mL; $P < 0.01$), whereas the TRT2 group tended to have a greater concentration of total IgG than the CTR group (17.47 ± 4.54 vs. 6.73 ± 3.43 mg/mL; $P = 0.07$). However, no difference was observed among treatment groups at 14 and 35 DIM.

There was also an interaction between treatment and week with regards to the concentration of Hp in the milk ($P < 0.01$; Fig. 6). At 3 DIM, cows in both the TRT1 and the TRT2 groups had a lower concentration of milk Hp than cows in the CTR group ($P < 0.01$), whereas no difference between the TRT1 and TRT2 groups was observed. At 14 DIM, milk Hp in the TRT1 cows was lower ($P = 0.03$) and that in the TRT2 cows tended to be lower ($P = 0.08$) than milk Hp in the CTR cows. No difference in concentration of Hp in milk was detected among treatment groups at 35 DIM.

The concentration of MAA was also affected by LAB treatment ($P = 0.02$) and DIM ($P < 0.01$; Fig. 7). The interaction between treatment and DIM had no significant effect on MAA. Overall, TRT1 cows (5.97 ± 0.96 μg/mL) had greater concentrations of MAA than both TRT2 (1.82 ± 1.04 μg/mL; $P < 0.01$) and CTR cows (3.02 ± 0.97 μg/mL; $P = 0.04$). However, the TRT2 group was not different from the CTR group with regards to MAA.

**Effect of Lactic Acid Bacteria Treatment on Milk Production**

Milk production is presented as the average daily milk during the first 8 wk after calving (Fig. 8). There was a significant interaction between treatment and parity regarding milk production ($P = 0.03$). Among multiparous cows, those in the TRT1 and TRT2 groups had greater milk production than cows in the CTR group ($P < 0.01$ and $P = 0.02$, respectively); TRT1 cows also produced greater amounts of milk compared with cows in the TRT2 group ($P = 0.03$). Among primiparous cows, those in the TRT2 group had greater milk production than the CTR cows ($P = 0.04$), whereas there were no differences between TRT1 and TRT2 or TRT1 and CTR cows.
The effect of LAB treatment on the ADFI during the first 8 wk after calving was dependent on parity \((P < 0.01;\) Fig. 9). Among primiparous cows, those in the TRT1 group had lower feed intake than those in the TRT2 \((P < 0.01)\) and CTR \((P < 0.01)\) groups; feed intake in the TRT2 group was not different from that of the CTR group. However, there was no difference among treatment groups in multiparous cows with regards to feed intake.

Feed efficiency, presented as the ratio of milk production to feed intake, was affected by the interaction between LAB treatment and parity \((P < 0.01;\) Fig. 10).

Among multiparous cows, both TRT1 \((P < 0.01)\) and TRT2 \((P = 0.02)\) enhanced feed efficiency compared with the CTR, but no differences were evidenced between the TRT1 and TRT2 groups. With respect to primiparous cows, the TRT1 group had the greatest feed efficiency, whereas the CTR group had the lowest and the TRT2 group was positioned in the middle, and all of them were different from each other \((P < 0.01)\). Body condition score was affected by both parity \((P < 0.01)\) and week \((P < 0.01)\) but not by LAB treatment (data not presented). Multiparous cows had lower BCS than primiparous cows \((P < 0.01)\).

In all
cows, BCS deceased from wk –1 to +9. The BCS was not affected by 2-way or 3-way interactions.

**Effect of Lactic Acid Bacteria Treatment on Milk Composition**

The effect of LAB treatment on milk composition is shown in Table 2. Both lactose ($P < 0.01$) and protein ($P = 0.05$) content differed among treatment groups. The content of lactose in the TRT2 group was greater than that in the TRT1 group ($P < 0.01$) and tended to be greater than that in the CTR group ($P = 0.05$). Milk from cows in the TRT1 group had a greater content of protein than the CTR group cows ($P = 0.02$), but none of them was different from cows in the TRT2 group. There was no effect of treatment on milk fat content, the ratio of milk fat to protein, milk urea nitrogen, and total solids of the milk or somatic cell count. It should be pointed out that all the aforementioned indicators
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continued with week except milk urea nitrogen. In addition, there was no interaction between treatment and week in terms of all milk components measured.

**DISCUSSION**

Previous research has shown that uterine infections immediately postpartum affect milk yield and composition during the first 100 DIM (Bell and Roberts, 2007). Moreover, in a companion article, we reported that the same intravaginal administration of LAB in the same cows lowered the incidence rate of metritis in the treated animals (Deng et al., 2015). Therefore, the objectives of this part of the study were to evaluate if LAB would also improve metabolic status and milk production and composition of the treated cows. Indeed, results showed that treatment with intravaginal LAB lowered the concentration of NEFA in the serum, increased milk IgG, lowered milk Hp, and, more importantly, improved milk efficiency. Details of the findings are discussed below.

Results of this study showed that intravaginal infusion of probiotics around calving was associated with lowered concentrations of NEFA in the serum of cows in the TRT2 group but not of cows in the TRT1 group. Nonesterified fatty acids are lipid metabolites that have been reported to increase in blood immediately after parturition. Moreover, elevated plasma NEFA have been associated with greater risk of metritis and the incidence of other periparturient diseases (Sordillo et al., 2009; Leblanc, 2010; Giuliodori et al., 2013). Because probiotics in our experiment were infused in the vaginal tract and not orally, the likely reason for lower plasma NEFA is that LAB lowered the incidence of uterine infections and boosted local production of secretory IgA in the vaginal mucus (Ametaj et al., 2014; Deng et al., 2015). Lactobacilli are known for their ability to prevent infections caused by pathogenic bacteria (Reid and Bruce, 2003; Doron and Gorbach, 2006). Several investigators have demonstrated that infection of the uterus is associated with translocation of endotoxins and proinflammatory cytokines into the blood circulation of dairy cows (Mateus et al., 2003; Kucharski et al., 2008). Moreover, Zu et al. (2009) reported that endotoxins stimulate lipolysis in adipose tissue and increase concentrations of NEFA in blood. Therefore, it is likely that the NEFA-lowering effect of intravaginally administered LAB in the TRT2 group might be related to lower uterine infection in the treated cows and, consequently, to lower endotoxin translocation into the systemic circulation. This is consistent with our previously published data, which suggest that postpartum inflammation and infection of the uterus are contributing factors to lipid alterations observed in transition dairy cows (Ametaj et al., 2005). It should be noted that there was a difference in the effects of TRT1 and TRT2 on serum NEFA immediately after calving (at wk 0), before the postpartum dose for TRT2 was administered. More research on vaginal bacterial composition of the 2 treatment groups might give insight into the potential factors that contributed to the difference observed.

Another interesting observation was that both groups of cows infused with probiotics in the vaginal tract exhibited greater concentrations of cholesterol in the serum compared with CTR cows. It is well known that cows that develop negative energy balance lose body condition as body reserves are mobilized and have lower circulating concentrations of cholesterol, glucose, and insulin and higher concentrations of fatty acids and urea compared with cows in positive energy balance (Gross et al.,

<table>
<thead>
<tr>
<th>Component</th>
<th>TRT1</th>
<th>TRT2</th>
<th>CTR</th>
<th>P-value</th>
<th>TRT × week P-value</th>
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<td>0.70</td>
<td>0.70</td>
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a,b Numbers within a row with different superscript letters are different at P < 0.05.

1TRT = intravaginal treatment of LAB or carrier.

2TRT1 = 2 consecutive LAB before parturition and 1 carrier dose after parturition, n1 = 20.

3TRT2 = 3 consecutive LAB doses around parturition, n2 = 20.

4CTR = 3 consecutive carrier doses around parturition, n3 = 20.

5From wk +1 to +5 on a weekly basis.

6MUN = milk urea nitrogen.

7TS = total solids.

8SCC = somatic cell count.
It has been recognized that infection and inflammation are associated with a variety of alterations in lipid metabolism (Feingold et al., 1992), including lowered concentrations of total cholesterol and lipoprotein cholesterol (Pruzanski et al., 2000). These changes in the plasma lipid composition are reflective of the host immune response, due to the ability of lipoproteins to bind several molecules that activate toll-like receptors through pathogen-associated molecular patterns, including endotoxins (Read et al., 1995), and the ability to serve as a scavenger for such molecules (Feingold et al., 1995). Greater cholesterol in the plasma of treated cows might indicate a lower translocation of endotoxins into the systemic circulation as less lipoprotein cholesterol had been utilized to neutralize endotoxins.

Another metabolite affected by LAB treatment was lactate. Cows in both LAB-treated groups maintained concentrations of lactate in the serum around calving whereas those in the CTR group showed an almost 30% decrease at 1 wk before parturition and remained at lower levels during the remaining experimental period. Of note, although plasma lactate was greater in cows treated with LAB, concentrations of lactate were within the normal values. Previously, we reported that the presence of fatty liver in transition dairy cows is negatively correlated with concentrations of lactate in the blood (Ametaj et al., 2005). Fatty liver affects almost 50% of dairy cows and has been related to endotoxemia and expedited removal of lipopolysaccharide–high density lipoprotein complexes to clean endotoxins from circulation (Ametaj et al., 2005). It is speculated that although infusion of LAB has no direct relationship to the metabolism of lactate, it is possible that LAB-treated cows might have he less endotoxins translocated into the circulation and suffered less from fatty liver and, therefore, kept the lactate concentrations in the blood from dropping. In support of this assumption are data indicating that skeletal muscles and liver consume more lactate during endotoxemic conditions, which might explain lower blood lactate in the control cows (Harada et al., 1994).

Lactic acid bacteria–treated cows had lower concentrations of Hp and greater concentrations of IgG in the milk at 3 DIM (i.e., colostrum) compared with their CTR counterparts. Lower milk Hp is indicative of better mammary gland health in LAB-treated cows compared with CTR cows (Grönlund et al., 2005). Haptoglobin is transported from blood into the milk but also originates from milk immune cells and epithelial cells in the mammary gland (Hiss et al., 2004). Additionally, greater milk IgG in the LAB-treated cows is important with respect to the health of the cow’s mammary gland and to the newborn calves, providing better protection against bacterial infections. In support of this finding are data that we previously reported in a companion article indicating elevation of secretory IgA in the vaginal mucus of the same cows treated with LAB (Deng et al., 2015). In alignment with greater milk IgG, cows in the TRT1 group had greater protein content in the milk than their counterparts in the CTR group, although those in the TRT2 group showed no differences. However, at 3 DIM, cows in the TRT1 group had greater MAA, an alternative indicator of the health of mammary gland, which was contradictory to the lower concentration of Hp. According to our clinical data, there was no difference between the LAB-treated cows and CTR cows with regards to subclinical mastitis based on somatic cell count in the milk (Deng et al., 2015); therefore, the reason underlying the divergent results needs to be further investigated.

Another important finding of this study was that LAB treatment increased milk production and feed efficiency in the treated cows. In a previous study, we observed that intravaginal infusion of the same probiotics for 6 consecutive weeks around calving increased milk production (Ametaj et al., 2014). Although the number of doses administered to cows in this study was only 2 to 3 per cow, we still observed greater milk production from both primiparous and multiparous cows. Probiotics studies in the past have demonstrated beneficial effects on milk yield, feed intake, and feed efficiency; however, they were mostly administered via the oral route (Krehbiel et al., 2003; Weinberg, 2003). In this study, the probiotic cocktail was applied intravaginally and still exhibited similar beneficial effects with respect to feed efficiency and milk production. Although the mechanism has not been thoroughly studied, there have been reports indicating that endotoxins and proinflammatory cytokines such as tumor necrosis factor inhibits hypothalamic centers related to feed intake (Becskei et al., 2008) and production of prolactin from the pituitary gland (Smith and Wagner, 1984; Hollis et al., 2005; Jana et al., 2005). Prolactin is a hormone known for its stimulatory effects on milk production, and its inhibition is associated with decreased milk production in dairy cows (Ollier et al., 2013). One of the potential mechanisms of how probiotics increased milk production in the current study could be related to prevention of uterine infections as well as translocation of endotoxins and proinflammatory cytokines into the blood circulation, which, compared with the CTR group, imposed less inhibitory effects on the release of prolactin and resulted in an increase in milk production. Therefore, it is likely that the differences in milk yield and milk composition among the LAB-treated cows and CTR cows are related to the overall improvement in the health status and metabolic profiles of those cows (Feingold et al., 1992; Markusfeld et al., 1997; Kida, 2003; Bell and Roberts, 2007).
Conclusions

Taken together, results of this study demonstrated that intravaginal infusion of LAB around calving modulated concentrations of selected metabolites in the serum of transition dairy cows as indicated by lower NEFA and greater cholesterol and lactate. Lactic acid bacteria treatment also was associated with increased total IgG and protein content in the milk as well as lower milk Hp. Moreover, cows treated with LAB exhibited greater milk production and improved feed efficiency. Although those findings are speculated to be related to reduction of uterine infections and endotoxin translocation into blood circulation, it would be interesting to look into the mechanisms by which intravaginal LAB influence milk production and the metabolic status of transition dairy cows. Also, intravaginal probiotics might have an unexploited potential to increase milk production in dairy cows.

LITERATURE CITED


