Iranian Journal of Applied Animal Science

Effect of a Multispecies Probiotics on Productive and Reproductive Performance of Holstein Cows

Research Article

Z. Merati[1,2] and A. Towhidi[1*]

[1] Department of Animal Science, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran
[2] Department of Animal Science, Faculty of Agriculture, University of Kurdistan, Sanandaj, Iran

Received on: 8 Jan 2021
Revised on: 21 Jun 2021
Accepted on: 15 Jul 2021
Online Published on: Jun 2022

*Correspondence E-mail: atowhidi@ut.ac.ir

Online version is available on: www.ijas.ir

ABSTRACT

The aim of this study was to evaluate the effect of supplemental multispecies probiotics on milk production and reproductive performance of postpartum lactating Holstein cows. Ninety-six cows were assigned to one of two dietary treatments from day 1 to 85 postpartum. Treatments were including 1) control, a standard diet, (n=48) and 2) probiotic, same as control plus 3 g/cow/day of the probiotics supplement (Hypro-cow®) (n=48). In a subset of eight animals per group, dry matter intake (DMI), body condition score (BCS), rectal temperature (RT), respiration rate (RR), and heart rate (HR) were assessed, and blood samples and milk yield recorded. Milk samples were analyzed every 2 wk. for fat, protein and somatic cell. Blood samples were collected on day 0, 14, 28, 42, 56, 70 and 85 post-partum for determining plasma total protein, albumin, creatinine, urea, glucose and triglyceride concentrations and activity of transaminases (aspartate aminotransferase and alanine aminotransferase). In all animals, occurrence of health disorders (i.e. metritis, endometritis, laminitis and mastitis), calving to first estrus interval, days open (DO), conception rate to first insemination and pregnancy up to 85 and 120 DIM were evaluated. Probiotic supplementation increased DMI (17.43±0.11 *vs.* 14.12±0.17) and BCS (3.36±0.16 *vs.* 3.14±0.23) ($P<0.05$). Mean daily milk yield (36.34±0.32 *vs.* 34.36±0.38) ($P=0.05$), and all milk components increased ($P<0.05$) by probiotic supplementation. Plasma concentrations of urea (10.28±0.63 *vs.* 11.08±0.47) and creatinine (1.00±S0.25 *vs.* 1.23±0.63) was lower ($P<0.05$) in supplemented group compared to control group. The incidence of laminitis (20.5±0.61 *vs.* 27±0.31) and metritis (32±0.03 *vs.* 40±0.81) were reduced ($P<0.05$) in supplemented cows compared to control cows. Interval from calving to first estrus and days open (DO) was reduced by 4 and 26 days, respectively ($P<0.05$) in treated cows. The conception rate was greater in probiotic group (22.5%) than control group (12.5%)**.** In conclusion**,** supplemental multi-species probiotics during postpartum has a beneficial effect on productive and reproductive performance of dairy cows.

KEY WORDS cattle, lactation, pregnancy, probiotics.

INTRODUCTION

Probiotics have been defined as “a feed supplement of live microorganisms that can improve the host animal condition upon ingestion through balancing the intestinal microbiome” (Alayande *et al.* 2020). Most commercial probiotics are lactic acid producing bacteria (e.g. *Lactobacillus* and *Streptococcus* spp.) or *Bacillus subtilis*. The use of *Lactobacillus* spp. culture may prevail the imbalances due to dietary alteration in the rumen of high yielding dairy cows during early lactation by promoting a more consistent level of lactic acid production to rumen microbiota which allows lactate utilizing bacteria to sustain a metabolically active population (Nocek *et al.* 2003). It has been reported that cows supplemented with 10^9 CFU of *L. acidophilus* per cow enhanced milk production by 1.8 kg in comparison to unsupplemented cows without affecting milk composition (Tesfaye and Hailu, 2019).

Bacillus subtilis that used as a probiotic is a transitory microorganism of the gastrointestinal tract and non-pathogenic to animals. The dietary *Bacillus subtilis* can pass through the stomach (Cutting, 2011) with the potential to be more resistant to the low pH. *Bacillus subtilis* could enhance an aerobiosis in the gastrointestinal tract, which favors native growth of *Lactobacilli* capable of producing lactic acid and inhibiting pathogenic bacteria proliferation (Souza *et al*. 2017).

Considering reproductive tract, vaginal lactobacilli prevent the proliferation of genitourinary pathogenic microorganisms through mechanisms of competitive exclusion of pathogens, stimulation of the host immunity, and production of specific antimicrobial compounds. Reid *et al*. (2001) reported that a combination of lactic acid bacteria strains decreased pathogens population in the vagina even when administered orally. In woman, a dietary dose of 10^8 viable probiotic lactobacilli per day could restore and keep urogenital health. Although a couple of antibiotics have been used to treat uterine infections in cattle, concerns about drug residues in the milk and bacterial acquisition of antibiotic resistance have prevented their widespread use (Otero *et al*. 2006). The administration of probiotics has been evaluated as a potential treatment for vaginitis in human (Reid and Bruce, 2003). Therefore, the similar mechanism may function in cows.

Beneficial effects of yeast culture on milk yield and composition (Dann *et al*. 2000) and rumen environment have been reported in dairy goat (Maragkoudakis *et al*. 2010). Recently, we have shown that supplemental *Saccharomyces cerevisiae* in postpartum lactating cows during the hot season, improved productive and reproductive performance, and immune function (Nasiri *et al*. 2018; Nasiri *et al*. 2019). Unlike, numerous trials have reported no effect of yeast on milk yield and composition (Biricik and Yavuz, 2001) in dairy cows. Because of these findings, using yeast with other probiotics might be necessary to see its cooperative effect on milk yield and composition. Komari *et al*. (1999) conducted a study using *L. acidophilus* and *Saccharomyces cerevisiae* culture together to investigate their effects on the performance of dairy cows and found out that daily 10 g yeast + 10 mL *L. acidophilus* treatment increased milk yield and milk fat content without affecting milk protein and lactose content. However, Campanile *et al*. (2008) indicated that supplementation of *Saccharomyces cerevisiae* did not affect milk yield and milk composition in buffalo cows. These inconsistent reports suggest that further studies need to know more about use of supplemental probiotics in lactating dairy cows.

It has been well documented that heat stress affects milk yield and composition, health status and reproductive performance in lactating cows. Under the heat condition, cows alleviate dry matter intake (DMI), spend less time exhibiting estrous and conserve energy needed to expel heat. They usually consume slug feed, sort feed and tend to choose feeds that do not produce as much heat during digestion such as grains (Samal, 2013; West, 2003; Sejian *et al*. 2012). Acidosis of rumen might be occurred by these behaviors. Slug feeding, lower levels of forage intake and higher levels of fermentable carbohydrates promote acids production and decrease rumen pH. Responsible bacteria for digestion of fiber are the most influenced at lowered pH of rumen (<6.0). These factors could reduce feed intake, milk production and often milk components (especially milk fat). Probiotics are bioregulators that can improve pH of rumen by promoting the use of lactic acid by the other bacteria (Nocek *et al*. 2003).

During the last decades, there are reports of beneficial effect of supplementing the animal feed with lactic acid producing bacteria on milk yield and reproductive performance (Souza *et al*. 2017). Also, the addition of yeast culture in the diet of Holstein cows was beneficial in improving milk production and milk fat, and some biochemical parameters of blood (Wohlt *et al*. 1998). There is a lack of data in the literature about the effects of yeast accompanied with other probiotics dietary supplementation on reproductive performance, milk yield and composition in dairy cows during heat stress. The present study, therefore, aimed to investigate the effects of a supplemental multispecies probiotic that consisted of *Saccharomyces cerevisiae*, *Bacillus subtilis*, *Bacillus lichenformis*, *Enterococcus faecium*, *Lactobacillus acidophilus*, *Lactobacillus planetarum*, *Bifidobacterium tedium*on milk yield and composition, some blood parameters, health status, and reproductive performance in postpartum lactating Holstein cows.

MATERIALS AND METHODS

Animal welfare

The Animal Care and Use Committee of the University of Tehran approved all experimental procedures of present study that performed according to international guidelines.

Animals and experimental design

This study was conducted in a large commercial dairy farm located in Kermanshah province, West of Iran during June to October 2017. The mean temperature-humidity index (THI) was 78.05 based on Yousef (1985) (Table1):

THI= T + 0.36 × DP + 41.2

Where:
T: temperature (˚C).
DP: dew point (˚C).

Stratified by predicted calving date, milk production in previous years, and body weight (BW). Ninety six Holstein cows (n=48) (mean±SD, BW=743±20.5 kg; 3.2±0.69 parities) were assigned at random to one of two dietary treatments: 1) a control diet or 2) control diet plus 3 g/day/cow of a recently developed probiotic supplement containing 5 × 10^8 CFU/g (Hypro-cow$^@$,Biorun LTD, Karaj, Iran). The probiotic supplement contained *Saccharomyces cerevisiae*, *Bacillus subtilis*, *Bacillus lichenformis*, *Enterococcus faecium*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Bifidobacterium tedium* and calcium carbonate. The hypro-cow probiotics supplement was well mixed with the diet immediately before each feeding. Cows were fed the dietary treatments three times a day (7:00, 13:00 and 19:00) from 1 to 85 d postpartum. Ingredients and chemical composition of cows' diet are shown in Table 2. Cows were housed under tie stalls and milked thrice per day at 5:00, 11:00, and 17:00.

Body condition score (BCS) and feed intake

In a subset of eight animals per group, feed intake was recorded individually from one day after calving until 85 d post-partum. The amount of feed given to each cow was weighed before feeding and the refused feed was weighed before the next feed. BCS was assessed by a trained technician at d 0, 20, 40, 50 and 85 postpartum, using a 5-point scale (1=thin and 5=fat).

Rectal temperature (RT), respiration rate (RR) and heart rate (HR)

RT using digital precision thermometer (TRD, Ellab Cropcopen Hagen, Denmark) were recorded daily at 11:00 h from one day after calving until 85 days after parturition. Also, RR (times/min) by counting the movements of flank for one minute and HR (times/min) of the tail vein were assessed three times per week during trial (Srikandakumar *et al*. 2003).

Milk sampling and analysis

Cows were milked three times daily at 02:00, 10:00, and 18:00 h. Milk weights were recorded at each milking for individual cow using a Waikato MKV milk meter (Inter Ag, Hamilton, New Zealand). Weekly milk samples were obtained postpartum from 3 consecutive milkings from each cow pooled and stored in 100-mL sterile tubes containing potassium dichromate until analysis.

Milk constituents including fat, protein, lactose, total solid, and somatic cell count (SCC) were analyzed using a Foss Milko-Scan (Foss Electric, Hillerød, Denmark). Fat corrected milk (FCM 3.5%) and energy corrected milk (ECM) was calculated according formula below. Calculated according to NRC (2001) equations:

3.5% FCM= (0.4324×kg of milk) + (16.216×kg of milk fat)
ECM= milk production × (0/383×% fat+0/242×% protein+0/7832) / 3.1138

Blood sampling

Blood was collected on day 0, 14, 28, 42, 56, 70 and 85 postpartum in eight animals per dietary treatment. Blood was collected from jugular vein by venipuncture before morning feeding. Blood was centrifuged in 1200 g for 15 min. Plasma samples were stored at -20 ˚C until analysis. Total proteins, albumin, creatinine, urea, triglyceride, glucose, aspartate aminotransferase- serum glutamic oxaloacetic transaminase (AST-SGOT) and alanine transaminase-serum glutamic pyruvic transaminase (ALT-SGPT) were assayed using the ZistChem® kits (Zist Chem diagnostics, 134 Tehran, Iran). Intra assay coefficients of variation were 8.4, 7.8, 8.2, 8.6, 9.1, 8.7, 8.1, 9.9 %, respectively.

Reproductive measurements

Observations for estrus were made at 06:00, 12:00, 18:00, and 24:00 hr. Cows that exhibited estrus sign (increased nervousness and activity, vaginal mucous discharge, mounting other cows, or standing estrus) in the morning were inseminated with commercially available frozen thawed semen in the evening, and cows that were in estrus in the evening were inseminated in the following morning. In cows that did not show estrus sign a 7-d OvSynch protocol was initiated 14 d after the second treatment with PGF_{2}-alpha also, timed-artificial insemination was performed 10 d later.

Ultrasonography (BCF Technology Ltd., Livingston, UK) was used to diagnosis pregnancy in 32-35 days post-artificial insemination (AI). The interval from calving to first AI (PPFSI), conception rate to fist AI, days open (DO), and pregnancy rate up to 85 and 120 d postpartum were recorded.

Health disorders

All (n=96) cows were observed for metritis daily from d 7 to 21 postpartum. Metritis was characterized by an abnormally large uterus and a reddish-brown vaginal discharge with fetid odor, accompanied with fever (>39 ˚C) and decreased feed intake and milk production.

Endometritis was diagnosed on 20-25 d postpartum as described by Sheldon *et al*. (2006).

Table 1 Meteorological data at Kangavar Station in the June to October 2017

Month	Max monthly temperature (˚C)	Min monthly temperature (˚C)	Average monthly temperature (˚C)	Average relative humidity (%)	Dew point (˚C)	THI[1] (%)
June	38.4	16.4	27.4	81.1	23.8	77.16
July	40.6	18.9	29.75	78.2	25.54	80.14
August	41.4	20.1	30.75	74.3	25.65	81.17
September	37.7	18.6	28.3	79.4	24.39	78.28
October	35.6	13.8	24.7	80.6	21.14	73.51

[1] THI: temperature-humidity index.

Table 2 Ingredients and chemical composition of the diet

Feed ingredients (% of dry matter)	Fresh	Early lactation
Barley grain	7.00	14.00
Corn grain	48.00	45.00
Soy bean meal	17.00	15.15
Canola meal	8.00	8.00
Full Fat	7.00	6.50
Vit-Min premix	1.50	0.90
Calcium- salt fat powder	0.00	1.60
CaCo3	1.00	1.30
NaHco3	1.60	1.90
NaCl	0.30	0.60
MgO	0.20	0.25
Fish meal	7.00	3.00
CH_4N_2O	0.00	0.40
Dicalcium phosphate	0.30	0.00
Toxin Binder	1.10	1.40
Chemical composition		
Crude protein (CP, %)	20.92	19.64
Net energy for lactation (NE_L) (Mcal/d)	1.80	1.84
Net energy for gain (NE_G) (Mcal/d)	1.20	1.29
NE/CP	0.086	0.094
CP/NE	11.596	10.663

From calving to 85 d postpartum, occurrence of lameness and mastitis were recorded. If a cow stood or walked in an abnormal gait, such as reluctance to bear weight on a hoof, or a noticeable limp with uneven steps, with or without the presence of a reddish, swollen or hot foot she was examined by a skilled veterinary practitioner who made the final diagnosis of laminitis. Mastitis was characterized by clinical signs (swelling, heat, hardness, redness, or pain in udder) and SCC in milk greater than 200000 cells/mL (Sharma *et al*. 2011).

Statistical analysis

The experiment was performed as a completely randomized design according to the following model:

$$Y_{ijk} = \mu + Ti + C_{j(i)} + Sk + (T\times S)_{ik} + e_{ijk}$$

Where:

Y_{ijk}: variable.

μ: mean.

T_i: fixed effect of treatment i.

Cj(i): random effect of cow j within treatment i.

Sk: time effect.

$(T\times S)_{ik}$: fixed effect of treatment-by-time interaction.

εijk: residual error.

Data were analyzed using Proc Mixed of SAS (Institute, version 9.1, 2002, Cary, NC, USA). The milk production in the previous lactation was included in the model as a covariate for the analysis of the milk production data. The BCS at calving was included in the model as a covariate for the analysis of BCS data. Dietary effect on binomial data were analyzed using logistic regression with the PROC GENMOD of SAS. Mean comparison of treatments were conducted with the Dunnett's test (P<0.05). Results are shown as mean ± SEM, unless indicated.

RESULTS AND DISCUSSION

Results presented in Table 3 showed significant difference in DMI between the probiotic group and control group from 24h after parturition to the end of lactation. DMI in probiotic group and control group increased linearly from d 1 to d 85 of parturition and was on average 15.31 ± 0.25 and

15.61 ± 0.61 kg/d in day 1 and 24.28 ± 0.74 and 22.31 ± 0.62 kg/d in day 85 after parturition, respectively. Overall, in probiotic group, DMI was significantly higher than control group (P<0.05).

Results regarding BCS during postpartum period (Table 3) show significant (P<0.05) increase in BCS in cows fed diet supplemented with probiotic as compared to control at the end of the experiment. The physiological responses of cows to treatments were expressed as changes in RR, RT and HR in comparison with the control group. Probiotic supplementation resulted in a slight, non-significant increases (P>0.05) in RR, RT and HR of cows during postpartum periods.

Results shown in Table 4 revealed significant effect of dietary supplementation of probiotic on milk yield and milk composition of Holstein cows. Average daily milk yield increased by about 3 kg in probiotic group as compared to control group. Milk production in two groups increased linearly from d 1 (32.74±1.22 *vs.* 32.51±0.89) to d 85 (36.01±0.25 *vs.* 33.15±0.19) of lactation, respectively (Figure 1a). The average of milk production from d 1 to d 42 was not significantly (P>0.05) different between two groups, whereas, from the d 42 to d 85, was significantly higher in probiotic group compared to control group. Moreover, dietary supplementation with probiotics significantly (P<0.05) increased milk components including percent milk fat (Figure 1b), milk fat content, percent milk protein (Figure 1c) and milk protein content compared to the control. Similarly, average fat corrected milk 3.5% and average ECM in probiotic group were higher than those in the control group. Mean SCC was lower in cows received the probiotics supplement. With the exception of the 24 hours following parturition, SCC was significantly lower (P<0.05) in the probiotic group than the control during whole experimental period (Figure 1d).

During postpartum period, results indicated that average total protein, glucose, albumin, triglyceride, SGPT and SGOT in plasma of cows were not significantly (P>0.05) affected by dietary probiotic supplementation. However, mean concentrations of urea-N and creatinine were significantly (P<0.05) decreased in supplemented group compared to control (Table 5). Plasma concentration of urea nitrogen and creatinine had a downward trend in both groups during the trial period (Figure 2a-b).

Data from Figure 3 show a significant decrease in percentage of laminitis and metritis in cows fed with probiotic compared to un-supplemented cows. However, consumption of probiotics had no significant effect on percentage of mastitis and endometritis cases in cows.

Data of reproductive performance indicated that the PPFSI was earlier by about 4d, and the DO was shorter by about 26 d in probiotic group than control group (59.20±1.64 *vs.* 63.88±1.06; 92.67±0.62 *vs.* 118.19±0.25, respectively) (Table 6). On the other hand, conception rate in first service and pregnancy rate at 85 and120 days were significantly (P<0.05) greater in the supplemented group compared to un-supplemented group (22.5±1.11 *vs.* 12.5±1.85; 62.5±0.09 *vs.* 42.5±1.22; 71.5±0.48 *vs.* 33.5±1.63, respectively).

Use of several probiotics have shown potential benefits in animal and human research trials. Responses in dairy cows have included effects on the milk yield and composition and reproductive performance (Nasiri *et al.* 2018; Nasiri *et al.* 2019). The aim of current study was to investigate efficacy of a newly developed multispecies probiotic (Hypro-cow) on production and reproduction performance in dairy cattle. In this study, supplemental probiotic Hypro-cow had a significant positive effect on feed intake and body condition score in postpartum cows. Similar results were indicated by Dann *et al.* (2000) during 5^{th} week of lactation (peak lactation), and cows that were supplemented with *Saccharomyces cerevisiae* maintained weight and body score better than controls, this could be attributed to improved DMI and improved gastrointestinal tract health. In agreement with the present result, some authors observed an improvement in feed intake when suckling calves were fed yeast and other strains of bacteria (Dann *et al.* 2000; Dehghan *et al.* 2020). In addition, similar results were reported on lactating buffaloes fed Biovet as micro-organisms added to their diets (Gujjar *et al.* 2006). Feeding yeast and multi-strain bacteria products may be most beneficial to dairy calves because of their effects on fermentation of rumen and digestive tract function in terms of increasing the digestibility of crude protein and acid detergent fiber (ADF) (Laborde, 2008). Different strains of bacteria can utilize residual oxygen introduced into the rumen with feeds, thus maintain an anaerobic environment (Calsamiglia *et al.* 2006). Specifically, after transition through the rumen, these organisms thrive in the intestine and use a great amount of carbohydrate for their proliferation and produce the relevant digestive enzymes (Giorgio *et al.* 2010). In addition, probiotics may alter the structure of gut epithelium and improve nutrient absorption by increasing area of absorptive surface (Giorgio *et al.* 2010).

In this study, supplemental probiotic significantly increased daily milk yield, FCM 3.5% and ECM. Moreover, review of milk production curves has shown that cows fed probiotics had greater peak of lactation compared tocontrol. These beneficial influences of feed additive on the peak lactation is also shown by Bryszak *et al.* (2019) in dairy cattle. Similar results were reported by Mostafa *et al.* (2014), for dairy cows fed two- probiotics (commercial yeast culture (*S. cerevisiae*) namely BGY 35 or a product of lactic acid bacteria and enzymes namely AVI-BAC®).

Table 3 Effect of dietary supplementation of probiotics on dry matter intake and body condition score (LS means) of cows at postpartum periods

Parameters	Probiotics	Control	P-value	SEM
Dry matter intake (kg/d)	17.43^{a}	14.12^{b}	0.04	0.06
Body condition score	3.36^{a}	3.14^{b}	0.0002	0.13

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Effect of dietary supplementation of probiotics on daily milk yield and chemical composition of milk (LS means) produced by cows at postpartum period

Parameters	Probiotics	Control	P-value	SEM
Daily milk yield (kg)	36.34^{a}	34.36^{b}	0.0001	0.84
FCM 3.5%	38.9^{a}	34.22^{b}	0.0001	0.62
ECM	36.04^{a}	32.72^{b}	0.0001	1.47
Milk fat %	3.55^{a}	3.21^{b}	0.0001	0.18
Milk fat content	144.99^{a}	121.16^{b}	0.002	8.21
Milk protein %	2.70^{a}	2.65^{b}	0.0001	0.02
Milk protein content	113.5^{a}	102.84^{b}	0.0003	4.1
Somatic cells count	176.24^{b}	187.13^{a}	0.0001	3.15

[1] Fat corrected milk (FCM 3.5%) and energy corrected milk (ECM) was calculated according the following formulas (NRC, 2001):
3.5% FCM= (0.4324×kg of milk) + (16.216×kg of milk fat) and ECM= milk production× (0/383×% fat+0/242×% protein+0/7832) / 3.1138.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

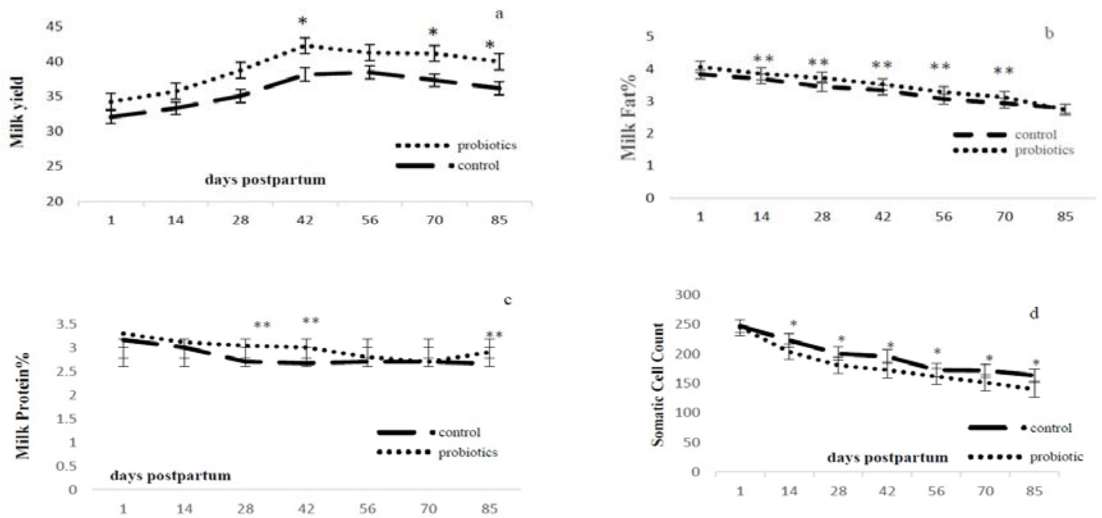

Figure 1 Change of LS means milk yield (a), milk fat percent (b), milk protein percent (c) and somatic cell count (d) in control or probiotics-fed lactating dairy cows during hot season
* (P<0.05) and ** (P<0.01)

Table 5 Effect of dietary supplementation of probiotics on blood parameters (LS means) of cows at postpartum period

Parameters	Probiotics	Control	P-value	SEM
Glucose (mg/dL)	64.37	59.04	0.15	5.26
Urea-N (mg/dL)	10.28^{b}	11.08^{a}	0.04	0.24
Creatinine (mg/dL)	1.00^{b}	1.23^{a}	0.0001	0.08
Total protein (g/dL)	6.02	5.71	0.94	0.58
Albumin (g/dL)	2.91	2.79	0.5	0.26
Triglyceride	9.16	9.57	0.76	1.22
SGOT	90.19	90.93	0.97	1.06
SGPT	7.21	6.91	0.64	2.12

SGOT: serum glutamic oxaloacetic transaminase and SGPT: serum glutamic pyruvic transaminase.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

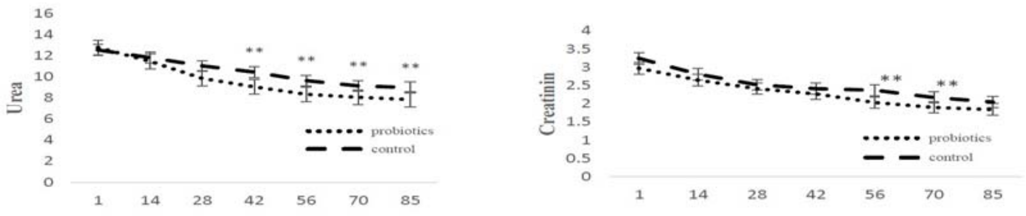

Figure 2 Change of LS means blood urea nitrogen (a) and creatinine (b) in control or probiotics-fed lactating dairy cows during hot season
** (P<0.01).

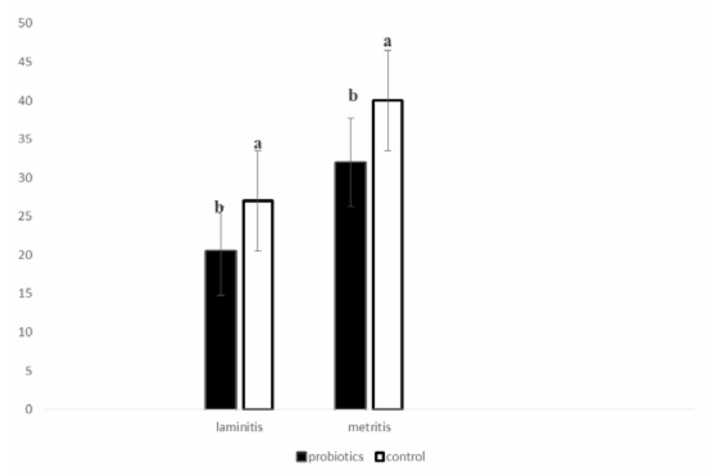

Figure 3 Effect of supplemental probiotics on health disorders (LS Means) of lactating cows during experiment
The means within the same bars with at least one common letter, do not have significant difference (P>0.05)

Likewise, Newbold *et al.* (1998) reported that relative increase in milk production in cows fed yeast culture was related to a change in DMI but linked to the complementation of bacterial strains in the feed with the native microbial flora and increased absorption of nutrients. The probiotics are thought to enhance the maintenance of the cellulolytic flora and improve the fiber degradation of plants. Like the change in DMI measured in our study, a similar change in DMI and milk production during postpartum was observed by Nocek *et al.* (2003) that diets supplemented with probiotics containing yeast culture and bacteria had beneficial effects on milk production, which increased by 2.3 kg/cow/day and that animals ingested more dry matter in the ration.

There was a tendency for greater milk fat content in cows receiving Hypro-cow probiotic as compared with control cows throughout the lactation weeks. *Saccharomyces cerevisiae* can stabilize pH of rumen through the competing with bacteria that produce lactate and allowing the bacteria proliferation that produce acetate and butyrate in the rumen that are precursors volatile fatty acids (VFA) for milk fat synthesis (Lesmeister *et al.* 2004). Iwanska *et al.* (1999) observed that increased milk production linked with supplementation with *Saccharomyces cerevisiae* 1026 increased milk production that was not always associated with variations in milk protein and fat content. Supplementation with direct-fed-microbial product consisting of two strains of *Enterococcus faecium* and *Saccharomyces cerevisiae* enhanced percentage of milk fat due to increased VFA production (Dutta *et al.* 2009).

Supplementation of early lactation dairy cows with probiotic altered the rumen fermentation patterns in favour of propionate, with potential benefits for energy balance and animal productivity (Aikman *et al.* 2011).

The stimulation of lactic acid utilising bacteria could account for *Saccharomyces cerevisiae*-induced decrease in lactic acid production (Beauchemin *et al.* 2003) and hence corresponding stabilization of ruminal pH. Stabilization of ruminal pH improves propionic acid production (Thrune *et al*. 2009).

In this study, plasma concentration of glucose, total protein, albumin, triglyceride, SGPT and SGOT were similar in the supplemented group and the control group, while blood urea nitrogen and creatinine concentrations were significantly decreased in cows fed probiotics in comparison with control. Probiotic supplementation was linked to significantly decreased plasma urea concentration from d 1 to 85 postpartum. The level of blood urea nitrogen is an indicator of using efficiency of dietary protein, degradability of protein in rumen and protein intake post rumen. Hence, supplemental probiotics may contribute in enhancement of the microbial protein synthesis and rumen efficiency in lactating cows (Ayad *et al*. 2013), probably due to better utilization of nitrogen from food in the rumen. The plasma urea-N concentration is related to the level of ammonia absorption from the rumen and/or the deamination of amino acids not deposited in the tissue (Lesmeister *et al*. 2004). Another possibility for the lower plasma urea-N concentration is that additives promote the utilization and deposition of nitrogen in tissues.

The reduction in blood creatinine we noted supplemented cows is in agreement with Ayad *et al*. (2013) and Lesmeister *et al*. (2004) who also reported that creatinine concentration were significantly lowered by feeding yeast culture in dairy cattle. In dairy cattle, creatinine is by product protein catabolism. Enhanced level of plasma creatinine indicates protein catabolism and degradation in the body. Supplemental probiotics strains may alleviate the negative energy balance and use of protein storage, therefore reduce concentrations of creatinine and urea in the blood (Lesmeister *et al*. 2004).

Also, we noticed an increase in creatinine with increased protein catabolism in control cows that is used as fuel in gluconeogenesis (gluconeogenic amino acids), for the manufacture of glucose, knowing although this period coincides with peak lactation, where a supply of glucose is required for the production of lactose.

A significant decrease in percentage of metritis and laminitis in cows fed with probiotic was shown in Table 7. Laminitis is a principal reason of lowered estrous signs in lactating cows (Rathwell, 2000). Lameness is associated with severe economic detriments, for example, lowered milk production, body weight loss, enhanced veterinary costs, delayed or silent estrous, increased days open, and early culling related with genetic potential loss (Rathwell, 2000). Multiple factors are associated with this disorder, but nutrition is one of the major contributors (Rathwell, 2000). It is likely that probiotics reduced lameness by modifying the rumen structure and pH stability.

Infections of uterus negatively influence postpartum dairy cows lowering the reproductive and productive performance. Some studies have reports that the principal reason for culling of lactating dairy cows is poor reproductive performance (Ametaj *et al*. 2014). The usage of probiotics in the reproductive tract can prevent the undesirable outcomes of the antibiotics routinely administered in the treatment of infections (Otero *et al*. 2006). The dairy cow vagina is usually populated by a bacteria diversity dominated fundamentally by lactic acid bacteria (Deng *et al*. 2015).

Lactic acid bacteria, mostly lactobacilli, have been utilized to treat infections of urogenital tract in women. It has been reported that lactobacilli strains of probiotics, specifically selected for their capability to prevent proliferation of urogenital tract pathogen, may colonize the vagina after orally feeding and remained for several months.

This means that the microorganisms successfully survived in the low gastric pH and bile salt, and passage through the intestinal tract, and that they then ascended without functional intervention, into the vagina. Therefore, oral delivery can be used to convey health benefits to reproductive tract. Lactobacilli strains of probiotics are acid and bile tolerant, but it has not been elucidated what mechanisms they applied to colonize the vagina and intestine (Reid *et al*. 2001). However, diminished metritis in treated dairy cows in current study might be related to similar mechanism in humans. Further studies need to clarify this matter.

Result of reproductive performance show that there was beneficial effect of probiotic supplementation on reproductive parameters of dairy cattle that is in agreement with results of Mostafa *et al*. (2014). The authors noticed improvement in the reproductive performance of cows after yeast probiotics supplementation. In the current study, cows supplemented by probiotic, had shorter interval between the first inseminations and the days open, while higher conception rate. We have recently reported cows fed diets supplemented with live yeast had greater plasma insulin like growth factor (IGF) -I, 17 β-estradiol and progesterone concentration, larger ovulatory follicles, shorter estrus cycles and improved reproductive performance (Nasiri *et al*. 2018). Therefore, the probiotic Hypro-cow that consisted live yeast and multispecies strains including *Saccharomyces cerevisiae* may improve reproduction in cows with similar mechanisms. Abdel-Khalek (2003) reported that *Saccharomyces cerevisiae* supplementation improved PPFSI, DO and service period in multi-parous Friesian cows. This finding agrees with some previous experiments referring to a reduction in the rate of service/conception and improvement in the conception rate related to supplemental live yeast in dairy cows (Moallem *et al*. 2009).

Table 6 Effect of dietary supplementation of probiotics (X±SE) on average reproductive performance of cows at post-partum periods

Parameters	Open days	PPFSI	Conception rate in first insemination (%)	Conception rate within 120 days post-partum (%)
Probiotics	92.67[b]	59.2[b]	22.5[a]	62.5[a]
Control	118.19[a]	63.88[a]	12.5[b]	42.5[b]
P-value	0.0001	0.05	0.04	0.002
SEM	3.57	1.66	0.47	0.44

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 7 Effect of dietary supplementation of probiotics (X±SE) on average index health of cows at post-partum periods

Parameters	Mastitis %	Laminitis %	Metritis %	Endometritis %
Probiotics	14	20.5[a]	32[a]	10
control	12.5	27[b]	40[b]	12
P-value	0.13	0.05	0.03	0.5
SEM	2.34	1.12	0.87	1.06

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

A potent relationship between the negative energy balance (NEB) amount in early lactation period and lowered pregnancy rate has been documented (Laborde, 2008), which may show improving energy balance of animals supplemented with probiotics, reflecting augmentation in reproductive indices of treated cows compared to the control cows. In addition, the effect of NEB during early lactation on later reproductive performance is well documented, acting via disruption of the hypothalamus-pituitary-ovary axis (Butler and Smith, 1989). Both the duration and magnitude of NEB have been associated with increased concentrations of growth hormone and decreased concentrations of insulin and IGF; directly reducing follicular competence and its response to circulating gonadotrophins (Lucy, 2001; Butler and Smith, 1989). Furthermore, NEB has been linked with delaying and reducing the magnitude of the LH surge, resulting in delayed resumption of luteal activity, increased incidence of cystic ovarian disease, and a lower probability of pregnancy to first insemination (Ospina *et al*. 2010; McArt *et al*. 2012). In this experiment, conception rate increased in cows fed probiotic. Therefore, multispecies probiotics supplementation seems attenuate the harmful impacts of negative energy balance on reproductive performance of dairy cows.

CONCLUSION

It can be concluded that feeding multispecies probiotics Hypro-cow increased milk yield, and milk fat and protein percentage. Supplemental probiotics also decreased health disorders including laminitis and metritis. Reproductive performance was improved in cows fed probiotics. Because each probiotic is based on distinct microbial species or strains with unique characteristic and behavior, this is something that can illustrate various outcomes by other products. Further studies need to clarify the modes of action and effectiveness of different probiotics in dairy cows especially on reproductive tract.

ACKNOWLEDGEMENT

This study was conducted in the form of an internal study opportunity at University of Tehran, and carried out in Milk and Livestock Company of Kangavar, Ferdows Pars Farming Holding Functions. The authors thank the managers of farm (Mr. Shafiee and Mr. Pakdel) for providing animals and facilities. We also appreciate Biorun Company, and MSRT of IRAN for financial support.

REFERENCES

Abdel-Khalek A.E. (2003). Productive and reproductive performance of primiparous and multiparous Friesian cows fed rations supplemented with yeast culture (Yea-Sacc1026). *Egyptian J. Nutr. Feeds*. **6,** 1095-1105.

Aikman P.C., Henning P.H., Humphries D.J. and Horn C.H. (2011). Rumen pH and fermentation characteristics in dairy cows supplemented with megasphaera elsdenii NCIMB 41125 in early lactation. *J. Dairy Sci*. **94,** 2840-2849.

Alayande K.A., Aiyegoro O.A. and Collins NjieAteba C.N. (2020). Probiotics in animal husbandry: Applicability and associated risk factors. *Sustainability*. **12(3),** 1087-1095.

Ametaj B.N., Iqbal S., Selami F., Odhiambo J.F., Wang Y., Ganzle M.G., Dunn S.M. and Zebeli Q. (2014). Intravaginal administration of lactic acid bacteria modulated the incidence of purulent vaginal discharges, plasma haptoglobin concentrations, and milk production in dairy cows. *Res. Vet. Sci.* **78,** 158- 166.

Ayad M.A., Benallou B., Saim M.S., Smadi M.A. and Meziane T. (2013). Impact of feeding yeast culture on milk yield, milk components, and blood components in Algerian dairy herds. *J. Vet. Technol. Sci.* **4,** 2-8.

Beauchemin K.A., Yang W.Z., Morgavi D.P., Ghorbani G.R and Kautz W. (2003). Effects of bacterial direct-fed microbials and yeast on site and extent of digestion, blood chemistry, and subclinical ruminal acidosis in feedlot cattle. *J. Anim. Sci.* **81,** 1628-1640.

Biricik H. and Yavuz M. (2001). Effect of *Saccharomyces cerevisiae* yeast culture on milk production, milk composition and some rumen and blood parameters of dairy cows. *J. Vet.* **20,** 9-12.

Bryszak M., Szumacher-Strabel M., El-Sherbiny M., Stochmal A., Oleszek W. and Roj E. (2019). Effects of berry seed residues on ruminal fermentation, methane concentration, milk production, and fatty acid proportions in the rumen and milk of dairy cows. *J. Dairy Sci*. **102,** 1257-1273.

Butler W.R. and Smith R.D. (1989). Interrelationship between energy balance and postpartum reproductive function in dairy cattle. *J. Dairy Sci*. **72,** 767-783.

Calsamiglia S., Castillejos M. And Busquet M. (2006). Alternatives to antimicrobial growth promoters in cattle. *Rec. Adv. Anim. Nutr*. **19,** 129-167.

Campanile K.A., Zicarelli F. and Vecchio D. (2008). Effects of *Saccharomyces cerevisiae* on *in vivo* organic matter digestibility and milk yield in buffalo cows. *Livest. Sci*. **114,** 358-361.

Cutting S.M. (2011). *Bacillus* probiotics. *Food Microbiol*. **28,** 214-220.

Dann H.M., Drackley J.K., McCoy G.C., Hutjens M.F. and Garrett J.E. (2000). Effects of yeast culture (*Saccharomyces cerevisiae*) on prepartum intake and postpartum intake and milk production of Jersey cows. *J. Dairy Sci*. **83,** 123-127.

Dehghan M., Banadaky H., Rajaei Sharifabadi M. and Vazirigohar M. (2020). A meta analysis of the effect of probiotics administration on growth performance of Suckling calves in Iran. *Iranian J. Appl. Anim. Sci*. **10,** 213-219.

Deng Q., John F., Farooq U., Lam T., Suzanna M. and Burim N. (2015). Intravaginal lactic acid bacteria modulated local and systemic immune responses and lowered the incidence of uterine infections in periparturient dairy cows. *PLoS ONE*. **10(4),** e0124167.

Dutta T.K., Kundu S.S. and Kumar M. (2009). Potential of direct-fed-microbials on lactation performance in ruminants. *Livest. Res. Rural Dev*. **21,** 160-166.

Giorgio G., Nina C. and Yantyati W. (2010). Importance of Lactobacilli in food and feed biotechnology. *Res Microbiol*. **61,** 480-487.

Gujjar S.R., Ahmad M. and Javid R.S. (2006). Effect of biovet and probiotic (bm-technology) on milk production in lactating buffaloes. *Pakistan Vet. J*. **26,** 201-203.

Iwanska S., Strusinska D., Zalewski W. and Opalka A. (1999). The effect of *Saccharomyces cerevisiae* 1026 used alone or with vitamin-mineral premix on milk yield and milk composition in dairy cows. *Acta Vet. Hung.* **47,** 41-52.

Komari R.K., Reddy Y.K.L., Suresh J. and Raj D.N. (1999). Effect of feeding yeast culture (*Saccharomyces cerevisiae*) and *Lactobacillus acidophilus* on production performance of crossbred dairy cows. *J. Dairy Sci*. **82,** 128-139.

Laborde J.M. (2008). Effects of probiotics and yeast culture on rumen development and growth of dairy calves. MS Thesis. Louisiana State Univ., Baton Rouge, Louisiana.

Lesmeister K.E., Heinrichs A.J. and Gabler M.T. (2004). Effects of supplemental yeast (*Saccharomyces cerevisiae*) culture on rumen development, growth characteristics, and blood parameters in neonatal dairy calves. *J. Dairy Sci*. **87,** 1832-1839.

Lucy M.C. (2001). Reproductive loss in high-producing dairy cattle: Where will it end? *J. Dairy Sci.* **84,** 1277-1293.

Maragkoudakis P.A., Mountzouris K.C., Rosu C., Zoumpopoulou G., Papadimitriou K., Dalaka E., Hadjipetrou A., Theofanous G., Strozzi G.P. and Carlini N. (2010). Feed supplementation of *Lactobacillus plantarum* PCA 236 modulates gut microbiota and milk fatty acid composition in dairy goats—A preliminary study. *Int. J. Food Microbiol*. **141,** 109-116.

McArt J.A.A., Nydam D.V. and Oetzel G.R. (2012). Epidemiology of subclinical ketosis in early lactation dairy cattle. *J. Dairy Sci*. **95,** 5056-5066.

Moallem U., Lehrer H., Livshitz L., Zachut M. and Yakoby S. (2009). The effects of live yeast supplementation to dairy cows during the hot season on production, feed efficiency, and digestibility. *J. Dairy Sci.* **92,** 343-351.

Mostafa T.H., Elsayed F.A., Ahmed M.A. and Elkholany M.A. (2014). Effect of using some feed additive (two- probiotics) in dairy cow rations on production and reproductive performance. *Egyptian J. Anim. Prod*. **51,** 1-11.

Nasiri A.H., Towhidi A., Shakeri M., Zhandi M., Dehghan-Banadaky M. and Colazo M.G. (2018). Effects of live yeast dietary supplementation on hormonal profile, ovarian follicular dynamics, and reproductive performance in dairy cows exposed to high ambient temperature. *Theriogenology.* **122,** 41-46.

Nasiri A.H., Towhidi A., Shakeri M., Zhandi M., Dehghan-Banadaky M., Pooyan H.R., Sehati F., Rostami F., Karamzadeh A., Khani M. and Ahmadi F. (2019). Effects of *Saccharomyces cerevisiae* supplementation on milk production, insulin sensitivity and immune response in transition dairy cows during hot season. *Anim. Feed Sci. Technol.* **251,** 112-123.

Newbold C.J., Mcintosh F.M. and Wallace R.J. (1998). Changes in the microbial population of a rumen-simulating fermenter in response to yeast culture. *Canadian J. Anim. Sci*. **78,** 241-244.

Nocek J.E., Kautz P.W., Leedle J.A.Z. and Block E. (2003). Direct-Fed microbial supplementation on the performance of dairy cattle during the transition period. *J. Dairy Sci.* **86,** 331-339.

NRC. (2001). Nutrient Requirements of Dairy Cattle. 7th Ed. National Academy Press, Washington, DC., USA.

Ospina P.A., Nydam D.V., Stokol T. and Overton T.R. (2010). Associations of elevated nonesterified fatty acids and beta-hydroxybutyrate concentrations with early lactation reproductive performance and milk production in transition dairy cattle in the northeastern United States. *J. Dairy Sci.* **93,** 1596-1603.

Otero M.C., Morelli L. and Nader-Macias M.E. (2006). Probiotic properties of vaginal lactic acid bacteria to prevent metritis in cattle. *Lett. Appl. Microbiol.* **43,** 91-97.

Rathwell A. (2000). Lameness in a dairy herd. *J. Dairy Sci*. **66,** 694-710.

Reid G., Andrew W., Bruce M., Fraser N., Heinemann C.H., Owen J. and Henning B. (2001). Oral probiotics can resolve urogenital infections. FEMS Immunol. *Med. Microbiol*. **30,** 49-52.

Reid G. and Bruce A.W. (2003). Urogenital infections in women: Can probiotics help? *Postgrad. Med. J*. **79,** 428-432.

Samal L. (2013). Heat stress in dairy cows - reproductive problems and control measures. *Int. J. Livest. Res*. **3,** 1994-2004.

Sejian V., Naqvi S.M.K., Ezeji T., Lakritz J. and Lal R. (2012). Environmental Stress and Amelioration in Livestock Production. Springer, Germany.

Sharma N., Singh N.K. and Bhadwal M.S. (2011). Relationship of somatic cell count and mastitis: An overview. *Asian-Australasian J. Anim. Sci*. **24,** 429-438.

Sheldon I.M., Lewis G.S., Le Blanc S. and Gilbert R.O. (2006). Defining postpartum uterine disease in cattle. *Theriogenology*. **65,** 1516-1530.

Srikandakumar A., Johnson A.E. and Mahgoub O. (2003). Effect of heat stress on respiratory rate, rectal temperature and blood chemistry in omani and Australian merino sheep. *Small Rumin. Res*. **49,** 193-198.

Souza V.L., Lopes N.M., Zacaroni O.F., Silveira V.A., Pereira R.A.N., Freitas J.A., Almeida R., Salvati G.G.S. and Pereira M.N. (2017). Lactation performance and diet digestibility of dairy cows in response to the supplementation of *Bacillus subtilis* spores. *Livest. Sci.* **40,** 91-97.

Tesfaye A. Hailu Y. (2019). The effects of probiotics supplementation on milk yield and composition of lactating dairy cows. *J. Phytopharmacol*. **8,** 12-17.

Thrune M., Bach M., Ruiz-Moreno M.D., Stern D. and Linn J.G. (2009). Effects of *Saccharomyces cerevisiae* on ruminal pH and microbial fermentation in dairy cows: Yeast supplementation on rumen fermentation. *Livest. Sci*. **124,** 261-265.

West W. (2003). Effect of heat stress on production in dairy cattle. *J. Dairy Sci.* **86,** 2131-2144.

Wohlt J.E., Corcione T.T. and Zajac P.K. (1998). Effects of yeast on feed intake and performance of cows fed diets based on corn silage during early lactation. *J. Dairy Sci.* **81,** 1345-1352.

Yousef M.K. (1985). Stress Physiology in Livestock. Volume I. Basic Principles. CRC Press, Boca Raton, USA.