

ASSESSMENT IMPACT OF USING LOCALLY PRODUCED PROBIOTIC BACTERIA ON THE PRODUCTIVE AND REPRODUCTIVE PERFORMANCE OF HOLSTEIN DAIRY COWS

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ABSTRACT

The current work was conducted for assessment impact of using locally produced probiotic bacterial strains on the production and reproductive performance of Holstein dairy cows, those probiotic strains were isolated from saliva and fecal matter of suckling calves and rumen liquor of dairy cows. Thirty multiparous Holstein dairy cows at last 3 weeks of pregnancy were allotted to two groups (15 per each). 1st group was the control group (C), fed transition diet (close up diet then post calving diet) without probiotic supplement, 2nd group was the treated group (T), fed the same transition diets improved with 10⁶ CFU/kg DM feed of the produced probiotic supplement. Daily milk yield of each cow were recorded for 3 months postpartum (PP). Results revealed that cows in the treated group significantly ($P < 0.05$) lost less BCS till day 30 PP than control then they gained more score at 3 months than control, probiotic supplementation non significantly ($P \geq 0.05$) increased average DMI throughout the whole experiment., probiotic supplementation improved ($P \geq 0.05$) average milk-to-feed ratio throughout the whole experiment. Treated group had a significant ($P < 0.05$) increase in cholesterol and AST (Aspartate aminotransferase) levels than control. Milk yield non significantly ($P \geq 0.05$) increased during whole experimental period, moreover increased ($P \geq 0.05$) average milk production throughout the whole experimental period by about 7.3% compared to control, while probiotic supplementation non significantly ($P \geq 0.05$) increased average milk fat% and yield (kg/day) or average milk protein% and yield, while had no clear effect on average solid not fat, milk lactose and ash percentages or yield compared to control. The effect of probiotics on reproduction revealed that cows in treated group had shorter days open, lesser I.N, fewer repeat breeders and better uterine and cervical involution and better conception rate at 3rd insemination (CR3) than control. So it can be concluded that, adding the produced probiotics supplement to dairy cows ration improved cows transition period BCS, milk production, milk quality and had a positive effect on reproductive performance and postpartum uterine involution.

Key words: Probiotics bacteria, milk yield, milk quality and reproductive performance

INTRODUCTION

Dairy production is a challenging business. It is known that the main goal of dairying is producing milk, but a lot of factors can influence a cow's milk production (MP). These factors include: stressors from environment, housing environment, management practices, diet, cow genetics, and the health of the cow. Dairy managers use tools and management practices to minimize and/or prevent obstacles that may negatively affect milk production. One such tool is probiotic bacteria in the diet.

Fuller (1989) defined probiotics or direct feed microbial (DFM) as "preparations consisting of live micro-organisms or microbial stimulants which affect the endogenous microflora of the recipient. Adult dairy cattle are primarily fed probiotic bacteria from transition to mid lactation due to the stressors involved at those stages in production. There is in consistence between previous studies on its effect on milk production and milk constituents, Qiao *et al.* (2010) observed an increase in milk yield and milk protein for cows supplemented with *Bacillus licheniformis*, but not for *Bacillus subtilis*. Meanwhile Mongkolthanasak (2012) reported Positive effects of *Bacillus* species as increased milk yield, fat-corrected milk, and milk protein. Kritas *et al.* (2006) and Qiao *et al.* (2010) reported an even

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greater increase in milk yield of 3.1 and 3.2 kg/d with *Bacillus subtilis* natto.

In similar ways, many authors reported that probiotic supplementation affected significantly on milk protein (%) (Rossow *et al.*, 2018; Hossain *et al.*, 2014), milk lactose (%) (Nocek and Kautz, 2006; Hossain *et al.*, 2014) and SNF% (Hossain *et al.*, 2014). While, some authors reported no significant influence of probiotics on yield of milk protein % (Weiss *et al.*, 2008; Maamouri *et al.*, 2014), lactose % (Maamouri *et al.*, 2014; Stella *et al.*, 2007) and ash % (Maamouri *et al.*, 2014).

According to recent statistics the culling rate of dairy cows during 2013 reached 41.7% (Can West and Valacta, 2013). Among all the culling reasons, reproductive failure was rated as the number one, accounting for 15.4% of the total culling rate (Can West and Valacta, 2013). The occurrence of health problems during the transition period is clearly a major complexed factor for subsequent reproductive performance (Ferguson, 2001), resulting in additional economic losses, poor transitions, also result in milk income losses. It has been noticed that the DFM supplementation significantly improves the reproductive ability, conception rate and fertility (Abu ElElla and Kommonna, 2013) as well as the immune response (young, 2012).

Since the beneficial effects of probiotics are strain dependent, it has been suggested that combinations of different probiotic strains may be more effective than single strain probiotics (Timmerman *et al.*, 2004). The rationale for multiple organisms comes from potential synergistic actions. It seems that probiotic strains of animal origin due to "host specific effect" are more effective (Fuller, 1989), and there are some studies that show animals generally benefits from

probiotic microorganisms isolated from their own digestive tracts (Walter, 2005). There are limited researches in dairy cows fed multi-strains of probiotics from calves and dairy cattle origin, so, the purpose of this study was feeding probiotics (a laboratory produced probiotics), of calves and dairy cows origin, isolated from a farm to be used in the same farm, during the transition period of cows to evaluate its effect on cows' health, production and reproductive performance.

MATERIALS AND METHODS

This experiment was carried out at Sanad farm (private Holstein dairy farm in Damietta governorate – Egypt) during the period from September, 2016 to June 2017 to evaluate impact of using locally produced probiotic bacterial stains on the productive and reproductive performance of Holstein dairy cows.

Microbial strains and growth condition of the used probiotics: Fifteen probiotic isolates were isolated from calves saliva and feces and rumen liquor of dairy cows revealed on 3 bacterial strains characterized and identified to be propagated and used as a probiotic supplementation. Lactobacilli isolates were grown on MRS broth (Oxoid) while Streptococci isolates were grown on M17 broth (Difco), after that the broth media incubated for 24 h at 37 °C. The strains were activated two or three times in order to obtain high biomasses in the stationary phase.

Genetic identification of isolated and tested strains: The isolates were identified according to 16sRNA in Sigma Company (Germany) by the automated sequencer (Table 1).

Table 1: The genetic identification of the tested probiotics stains.

Probiotic strain	Isolation source	Isolation media
Lactobacillus farraginis strain NRIC 0676 16S ribosomal RNA gene, partial sequence, NR 041467.1	Calves Saliva and Feces, Rumen liquor	MRS- M17
Lactobacillus reuteri strain NBRC 15892 16S ribosomal RNA gene. Partial sequence, NR 113820.1	Calves saliva	M17
Lactobacillus rhamnosus strain NBRC 3425 16S ribosomal RNA gene, partial sequence, NR 113332.1	Calves Feces, rumen liquor	M17

Cows, experimental design and diets:

Thirty multiparous Holstein dairy cows at last 3 weeks of pregnancy were allotted to two groups (15 per each). Total mixed rations (TMR) were formulated to meet the predicated requirements for energy, protein, minerals and vitamins during pre-partum and postpartum periods according to NRC

(2001). Ingredient and chemical composition of the basal diets is presented in table (2). The first group was assigned to the control group without treatment, while the other group (treated group) fed on the basal diet with 10⁶ CFU/kg DM feed of the produced probiotic supplement.

Table 2: Ingredients (As-fed basis) and proximate analysis of the experimented rations (post calving diet).

Ingredients	Post-calving diet	Pre-calving diet
	As- fed basis (kg/head /day)	As-fed basis (kg/head /day)
Corn grain, ground, dry	8	4.84
Soybean meal 47%	3.5	2.33
Flax (Linseed whole lent)	1.5	1
Corn silage	20	25
Alfalfa hay	5	-
Barseem (Egyptian clover)	3.5	-
Sodium Bicarbonate	0.24	-
Magnesium oxide	0.04	.03
Di- basic calcium phosphate	0.04	.06
Salt	0.06	-
Calcium carbonate	0.1	-
Premix ¹	0.1	.03
NEW T-NIL®Dry ² (Antimycotoxin)	0.04	0.03
Total	42.1	33.45
Chemical analysis:		
CP (% of DM) ³	16.5	14.6
NEL (M. Cal. /kg. DM) ⁴	1.65	2.81
NDF (% DM) ⁵	28.4	28.3
ADF (% DM) ⁶	18.6	17
Forage NDF (% DM)	21.8	21.7
TDN (% of DM) ⁷	74	77
E.E (% of DM) ⁸	4.8	5.2
RUP (% of CP) ⁹	38.75	

1= (Vit. A 10000000 IU, Vit D3 2500000 IU, Vit. E 35000 mg , Biotin 1000 mg , Zinc 100000 mg, Mn 80000 mg, Cu 30000 mg, I 800 mg, Co 400 mg , Se 300 mg , Caco3 to 3 kg).

2=Sorbic acid 0.05%, Citric acid 0.75%, Calcium propionate 10.5%, Copper sulphate 5%, Inactivated yeast (*Saccharomyces Cerevisiae*) 2%, Sapiolite 41.7%, Bentonite 40%

3 = crude protein, 4= net energy for lactation, 5= neutral detergent fiber, 6=acid detergent fiber, 7= Total digestible nutrients, 8 = ether extract, 9= rumen undegradable protein

Feed intake and Body condition score:

Cows were fed a total mixed ration (where concentrates and roughages were mixed mechanically using mixer wagon in the farm), the two groups fed equal diets, cows were fed close up diet for three weeks till parturition then fed post calving ration till end of the experiment (12 weeks postpartum). Cows were fed the post calving diet in quantities to provide excess of the expected daily intake for ad libitum consumption with feed refusal (3-5% of the total diet), while the Pre-calving cows were given a restricted diet from the close up ration and fed rice straw for free choice, cows were given a free access to water. Feed consumption was recorded daily by weighing feeds offered to and refused per each group.

Body condition scores of animals were performed by one evaluator using visual examination and palpation at the beginning of the experiment, 3 weeks before parturition then at day of parturition and at 4th weeks PP and at 12th weeks post calving, it was evaluated as described by Surinder *et al.* (1987).

Sampling, measurements and analyses:

Samples from the experimental ration were taken periodically for proximate analysis three times each every one month. Milk samples were taken one time every month for analysis of Milk constituents using milk scan. Milk samples were taken to the National Research Center for analysis of Milk constituents by

mid infra-red spectroscopy with a milk scan 4000 (Biggs *et al.*, 1987). Samples are taken from the same quarter before and after milking. Whole blood samples for progesterone concentration were collected in the mid luteal phase of 1st and 2nd insemination (7-12 day after ovulation) and at day 20 from 1st and 2nd insemination.

Also blood samples were collected 3 months PP at four hours after morning feeding at 8:00 am from the jugular vein to determine the biochemical parameters (blood metabolites) (Cholesterol, Triglyceride, ALT, AST, total protein and albumin) spectrophotometrically measured (T80 UV/VIS Spectrometer, PG Instruments Ltd, UK).

Collected Samples were centrifuged at 3000 xg for 10 min. at 4°C, to harvest serum, samples were frozen at (-20°C) until required for analysis.

Analytical procedures:

Proximate analysis of experimental diets: Proximate analysis of dietary ingredients and experimental rations including dry matters (DM), crude protein (CP), ether extract (EE), ash and nitrogen free extract (NFE) were analyzed according to A.O.A.C. (2000). Neutral detergent fiber (NDF) and acid detergent fiber (ADF), were analyzed by Ankom 2000 Fiber Analyzer as described by Van Soest *et al.* (1991).

Blood biochemistry and hormonal assay: Blood serum biochemistry was carried out at labs of Animal Nutrition, Animal Production Department, Faculty of Agriculture, Ain Shams University. Blood serum samples were analyzed using commercial kits. Total plasma protein concentrations was determined as described by Cannon *et al.* (1974), albumin concentrations was determined using method of Doumas *et al.* (1971), Triglycerides and cholesterol were determined according to Stein *et al.* (1987),

Alanine amino transferase (ALT) and aspartate amino transferase (AST) activities were calorimetrically determined according to AST and ALT kits based on reaction of Young (1990). Progesterone in was determined by using ELISA (Autrere and Benson., 1976).

Reproductive measures: Reproductive tract of all cows will be examined by using ultrasound scanning at 22-29 days post-partum and at 38-45 days post-partum for examination of uterine evolution (cervix diameter, two uterine horns diameters). Conception rate at 1st, 2nd and 3rd service were calculated only for services accompanied by ovulation. Successful pregnancy will be defined by using ultrasound scanning at day 42 after mating. Insemination number per 1st and 2nd conception, percentage of repeat breeders, days interval from calving to 1st estrus and 1st insemination and days open will be measured. The detailed description of the reproductive performance evaluation is according to Westwood *et al.* (2000).

Statistical analysis: Obtained data were analyzed using the statistical package SPSS for Windows XP V15.0 (SPSS Inc., Chicago, USA, 2007). The significance of differences between treated samples was evaluated using Anova test.

RESULTS

Body condition score (BCS):

Bacterial probiotic supplementation of dairy cattle ration reduced BCS (table 3) at calving by about 11.9% while BCS of control group reduced by about 12.7% compared to BCS of the same group 3 weeks pre-partum. Moreover, bacterial probiotic supplementation reduced BCS at 4th weeks postpartum by about 3.9% while BCS of control group reduced by about 14.5% compared to BCS of the same group at calving.

Table 3: Effect of dietary bacterial probiotic supplementation on body condition score (BCS) of dairy cows.

Stage of lactation (weeks postpartum)	Treatment	
	Control	Probiotic supplementation
3 weeks pre-partum	4.03±0.059 ^a	3.52±0.063 ^b
At calving	3.52±0.059 ^a	3.10±0.055 ^b
4 weeks post-partum	3.01±0.056 ^a	2.98±0.052 ^a
12 weeks post-partum	3.21±0.044 ^a	3.18±0.059 ^a

Values are expressed as means ± standard error (SE). Means within the same row of different litters are significantly different at (P < 0.05).

Dry Matter intake (DMI):

No difference was observed by using bacterial probiotic in the lactating cows diet in dry matter intake (DMI) during first and 2nd four weeks postpartum compared to control, as shown in table (4). However, probiotic supplementation significantly

(P < 0.05) increased DMI during 3rd month postpartum by about 6.9% and non-significantly (P ≥ 0.05) increased average DMI throughout the whole experimental period by about 3.3% compared to control.

Table 4: Effect of dietary bacterial probiotic supplementation on dry matter intake (DMI Kg/day/cow) of dairy cows.

Stage of lactation (weeks postpartum)	Treatment	
	Control	Probiotic supplementation
0 – 4	18.55±0.45 ^a	18.71±0.30 ^a
5 – 8	20.17±0.54 ^a	20.43±0.71 ^a
9 – 12	24.36±0.55 ^b	26.05±0.44 ^a
0 – 12 (Average)	21.03±0.47 ^a	21.73±0.70 ^a

Values are expressed as means ± standard error (SE). Means within the same row of different litters are significantly different at (P < 0.05).

Milk production:

The results of milk production is summarized in table 5. It was observed that probiotic supplementation in lactating cows ration non significantly (P ≥ 0.05) increased milk yield during 1st, 2nd and 3rd month

postpartum by about 4.6%, 9.9% and 0.15% respectively, moreover increased (P ≥ 0.05) average milk production throughout the whole experimental period by about 7.3% compared to control.

Table 5: Effect of dietary bacterial probiotic supplementation on postpartum milk production (Kg/day/cow) of dairy cows.

Stage of lactation (weeks postpartum)	Treatment	
	Control	Probiotic supplementation
0 – 4	33.77±1.22 ^a	35.31±2.05 ^a
5 – 8	34.27±1.10 ^a	37.65±2.67 ^a
9 – 12	33.65±0.78 ^a	33.70±2.34 ^a
0 – 12 (Average)	33.08±0.98 ^a	35.49±2.97 ^a

Values are expressed as means ± standard error (SE). Means within the same row of different litters are significantly different at (P < 0.05).

Milk composition:

The effect of probiotic supplementation in dairy cattle ration on milk composition is presented in table (6). It was noticed that bacterial probiotic supplementation in dairy cows ration non significantly (P ≥ 0.05)

increased average milk fat% and yield (kg/day) or average milk protein% and yield by about (20.5% and 10.1%) and (1.5% and 3.7%) respectively, while had no clear effect on average solid not fat, milk lactose and ash percentages or yield compared to control.

Table 6: Effect of dietary bacterial probiotic supplementation on postpartum milk composition of dairy cows.

Items	Treatment	Stage of lactation (weeks postpartum)			
		0 -4	5-8	9-12	0-12 (average)
Solid not fat (SNF)					
SNF%	Control	8.66±0.12 ^b	8.69±0.19 ^a	9.42±0.24 ^a	8.93±0.16 ^a
	Treated	9.03±0.18 ^a	8.14±0.66 ^a	9.36±0.26 ^a	8.91±0.22 ^a
SNF yield (kg/day)	Control	2.93±0.09 ^a	2.99±0.12 ^a	3.15±0.09 ^a	3.02±0.07 ^a
	Treated	3.14±0.19 ^a	2.78±0.28 ^a	3.09±0.23 ^a	3.05±0.17 ^a
Milk fat:					
Fat%	Control	2.25±0.29 ^b	3.36±0.44 ^a	3.91±0.41 ^a	3.17±0.24 ^a
	Treated	3.56±0.32 ^a	2.72±0.43 ^a	4.90±0.40 ^a	3.82±0.20 ^a
Fat yield (kg/day)	Control	0.87±0.16 ^b	1.15±0.16 ^a	1.34±0.15 ^a	1.19±0.11 ^a
	Treated	1.24±0.13 ^a	0.93±0.13 ^a	1.65±0.21 ^a	1.31±0.09 ^a
Milk sugar (Lactose):					
Lactose%	Control	4.74±0.06 ^a	4.76±0.11 ^a	5.13±0.15 ^a	4.88±0.08 ^a
	Treated	4.78±0.18 ^a	4.46±0.36 ^a	4.94±0.18 ^a	4.72±0.16 ^a
Lactose yield (kg/day)	Control	1.61±0.05 ^a	1.63±0.07 ^a	1.72±0.05 ^a	1.65±0.04 ^a
	Treated	1.67±0.12 ^a	1.57±0.15 ^a	1.64±0.14 ^a	1.62±0.11 ^a
Milk protein:					
Protein%	Control	3.14±0.04 ^a	3.15±0.07 ^a	3.42±0.09 ^a	3.24±0.05 ^a
	Treated	3.35±0.14 ^a	2.76±0.22 ^b	3.67±0.27 ^a	3.29±0.13 ^a
Protein yield (kg/day)	Control	1.06±0.03 ^a	1.08±0.04 ^a	1.14±0.03 ^a	1.09±0.02 ^a
	Treated	1.16±0.07 ^a	1.02±0.11 ^a	1.23±0.13 ^a	1.13±0.07 ^a
Milk ash:					
Ash%	Control	0.66±0.01 ^a	0.67±0.02 ^a	0.73±0.02 ^a	0.69±0.01 ^a
	Treated	0.68±0.02 ^a	0.62±0.05 ^a	0.73±0.01 ^a	0.68±0.02 ^a
Ash yield (kg/day)	Control	0.22±0.01 ^a	0.23±0.01 ^a	0.24±0.01 ^a	0.23±0.01 ^a
	Treated	0.24±0.02 ^a	0.22±0.02 ^a	0.024±0.02 ^a	0.24±0.02 ^a

Values are expressed as means ± standard error (SE). Means within the same column of different litters are significantly different at ($P < 0.05$).

Milk-to-feed ratio:

Probiotic supplementation in dairy cattle ration non significantly ($P \geq 0.05$) improved milk-to-feed ratio (table 7) during 1st and 2nd month postpartum by about 3.8% and 8.9% respectively, while reduced ($P \geq 0.05$) milk-to-feed ratio during 3rd month of

lactation by about 6.5% compared to control. Moreover, probiotic supplementation improved ($P \geq 0.05$) average milk-to-feed ratio throughout the whole experimental period by about 4.3% compared to control.

Table 7: Effect of dietary bacterial probiotic supplementation on postpartum milk-to-feed ratio of dairy cows.

Stage of lactation (weeks postpartum)	Treatment	
	Control	Probiotic supplementation
0 - 4	1.82±0.09 ^a	1.89±0.11 ^a
5 - 8	1.69±0.07 ^a	1.84±0.15 ^a
9 - 12	1.38±0.04 ^a	1.29±0.10 ^a
0 - 12 (Average)	1.62±0.06 ^a	1.69±0.11 ^a

Values are expressed as means ± standard error (SE). Means within the same row of different litters are significantly different at ($P < 0.05$).

Blood parameters

Table (8) shows the means values of some blood serum units. It was observed that probiotic supplementation in dairy cattle ration had no significant effect on serum total protein, albumin,

AST and ALT concentrations compared to control. Moreover, probiotic non significantly ($P \geq 0.05$) increased serum triglycerides concentration by about 10.2% while, significantly increased serum cholesterol by about 62.3% compared with control.

Table 8: Effect of dietary bacterial probiotic supplementation on some blood serum biochemical parameters of dairy cows.

Items	Treatment	
	Control	Probiotic supplementation
Total protein (g/dl)	10.99 ±0.78 ^a	9.59±0.41 ^a
Albumin (g/dl)	3.27±0.46 ^a	3.54±0.23 ^a
Triglycerides (mg/dl)	102.46±11.65 ^a	112.89 ±13.32 ^a
Total cholesterol (mg/dl)	163.51±16.02 ^b	265.91 ±49.20 ^a
AST (μ/L)	15.25 ±2.30 ^a	20.92±3.99 ^a
ALT (μ/L)	21.35±2.06 ^a	18.36 ±1.44 ^a

Values are expressed as means ± standard error (SE). Means within the same row of different litters are significantly different at (P <0.05).

Reproductive performance:

Regarding reproductive performance of dairy cows (table 9), it was observed that days from calving to 1st insemination was earlier by about 1.5 days, number of insemination per conception was less by about 4.4% and days open (DO) was shorter by about

9.5 days of cows in probiotic treated group compared to control. Moreover, probiotic supplementation in dairy cows ration improved conception rate% and repeat breeders% by about 21.9% and 55.6% respectively compared to control.

Table 9: Effect of dietary bacterial probiotic supplementation on reproductive performance of dairy cows.

Items	Treatment	
	Control	Probiotic supplementation
Days from calving to 1 st insemination	60.61 ±3.46a	59.09 ±2.69a
Insemination No.	2.28± 0.32a	2.18 ±0.30a
Days open	93.06±7.57a	83.56±8.88a
Conception rate (%) at 1 st insemination	27	27
Conception rate (%) at 2 nd insemination	44	46
Conception rate (%) at 3 rd insemination	11	27
Average conception rate (%)	27.3	33.3
Repeat breeders (%)	18.0	10.0

Values are expressed as means ± standard error (SE). Means within the same row of different litters are significantly different at (P <0.05).

Serum progesterone concentration:

The results indicated that feeding lactating cows on diets supplemented by bacterial probiotic non significantly (P≥0.05) increased serum

progesterone concentration (table 10) after both periods of 1stinseminate and 2nd insemination compared to control.

Table 10: Effect of dietary bacterial probiotic supplementation on serum progesterone during postpartum periods of dairy cows.

Items	Days after insemination	Treatment	
		Control	Probiotic supplementation
Progesterone (ng/ml) of 1 st insemination	7-12	1.43 ±0.08 ^a	1.62 ± 0.24 ^a
	20	1.35 ± 0.38 ^a	1.44 ±0.19a
Progesterone (ng/ml) of 2 nd insemination	7-12	0.55 ± 0.30 ^a	0.58 ±0.26 ^a
	20	0.43 ±0.31 ^a	0.75±0.38 ^a

Values are expressed as means ± standard error (SE). Means within the same row of different litters are significantly different at (P <0.05).

Postpartum uterine involution:

Results presented in table (11) show that bacterial probiotic supplementation in dairy cows ration shorten the period required for postpartum uterine

involution through reduction of uterine cervix, uterine right and left horn diameters during 3rd to 4th weeks postpartum by about 21.6%, 7.9% and 22.1% respectively compared to control.

Table 11: Effect of dietary bacterial probiotic supplementation on postpartum uterine involution of dairy cows.

Items	Treatment	
	Control	Probiotic supplementation
Uterine cervix diameter/mm		
At 22 - 29 days postpartum	29.60 ± 1.45 ^a	23.21 ± 2.12 ^b
At 38 - 45 days postpartum	28.96 ± 0.87 ^a	25.04 ± 2.33 ^a
Uterine right horn diameter/mm		
At 22 - 29 days postpartum	24.60 ± 2.15 ^a	22.65 ± 2.07 ^a
At 38 - 45 days postpartum	21.81 ± 2.38 ^a	23.66 ± 1.86 ^a
Uterine left horn diameter/mm		
At 22 - 29 days postpartum	26.43 ± 2.44 ^a	20.58 ± 1.74 ^b
At 38 - 45 days postpartum	20.77 ± 2.72 ^a	20.89 ± 2.68 ^a

Values are expressed as means ± standard error (SE). Means within the same row of different litters are significantly different at ($P < 0.05$).

DISCUSSION

Body condition score

Results revealed that cows in the treated group lost less BCS till day 4th weeks postpartum than control then they gained more score till 3rd month postpartum than control did, These results indicate that treated cows had better transition period and lower negative energy balance during the transition period which can be explained with Nocek *et al.* (2003) who showed that during the transition period cows receiving DFM had a higher concentration of blood glucose and insulin and a lower concentration of both β -hydroxybutyrate (BHBA) and non-esterified fatty acids (NEFA) when compared with the control cows. And agree with another study (Peng *et al.*, 2012) who reported lower plasma NEFA after calving with cows that were fed *Bacillus subtilis* natto. Lower concentration of NEFA indicates that cows are mobilizing less energy from adipose deposits to meet their high energy requirements. The decrease in BHBA levels indicate more efficient use of nutrients for production and a decrease in negative energy balance (Drackley, 1999). Also Luan *et al.* (2015) observed less subclinical ketosis after calving with cows consuming *Bacillus pumilus* as a DFM. Less ketones and ketosis suggest that supplementing probiotics can decrease the amount of energy that cows take from adipose tissue. When blood glucose is made available and cows are mobilizing less fatty acids from adipose tissues, glucose can route to the mammary gland to produce more milk.

Milk production and composition:

Response of average lactation performance (+7.3%) throughout the whole experimental period in bacterial probiotics treated group compared to control is of large biological significance and would result in favorable ratio of extra income to probiotic cost.

Modern dairy farms are targeting high milk production utilizing feed composed of high concentrates to meet the metabolic demand of the higher milk production. Such feeding system is associated with metabolic dysfunction like rumen acidosis especially during poor feeding condition and composition. Therefore, probiotics are suggested as an effective mechanism of preventing or treating ruminal acidosis and/or improve animals' performances (Lettat *et al.*, 2012). Thus, according to Nocek and Kautz (2006) the supplementation of probiotics (i.e. Yeast and *Enterococcus* mix, *Propionibacterium* (strain-P169), to dairy cows have significantly improved milk yield in comparison to control. Also, Yasuda *et al.* (2007) reported 3-16% increase in milk production in dairy cows by supplementing the diet with probiotics. Peng *et al.* (2012) who reported an even greater increase in milk yield of 3.1 and 3.2 kg/d with *Bacillus subtilis* natto.

Like milk yield, the effects of probiotics in milk composition were also inconsistent across different literatures. Consequently (Vibhute *et al.*, 2011; Rossow, 2018) were reported significant improvement in milk fat % after supplementation of probiotics. In contrary, Dutta and Kundu, 2008. In similar ways, different authors reported that probiotic supplementation have significantly affected milk protein (%) (Rossow *et al.*, 2018; Hossain *et al.*, 2014; Yalcin *et al.*, 2011; Vibhute *et al.*, 2011), milk lactose (%) (Nocek and Kautz, 2006; Hossain *et al.*, 2014) and SNF% (Hossain *et al.*, 2014). While, some authors have showed that, there was no significant influence of probiotics on yield of milk protein % (Nocek and Kautz, 2006; Weiss *et al.*, 2008; Maamouri *et al.*, 2014), lactose % (Weiss *et al.*, 2008; Maamouri *et al.*, 2014; Stella *et al.*, 2007) and Ashes % (Maamouri *et al.*, 2014).

Dry matter intake and milk-to-feed ratio:

Bacterial probiotic supplementation numerically improved DM intake and milk-to-feed ratio during postpartum period compared with control. Supports these findings Qiao *et al.* (2010) and Souza *et al.* (2017) observed no response to *Bacillus subtilis* on intake of lactating cows over a 10-week period. In addition, similar results were reported on lactating buffaloes fed Biovet as micro-organisms added to their diets (Gujjar *et al.*, 2006) or dairy goats fed Lecture as a bacterial feed additive (Ahmed *et al.*, 2008). However, Aikman *et al.* (2008) observed no difference in DM intake between the control and treated cows fed two TMR's differing in level of concentrate and supplemented with direct-fed microbial (DFM) during the first 14 weeks of lactation.

Biochemical Blood parameters:

Blood biochemical parameters are commonly used to assess the nutritional and physiological status of lactating animals (Kafilzadeh *et al.*, 2019). Results declared that treated group had a higher cholesterol and AST concentrations than control even they are within normal range. In agreement with these results, Mousa *et al.* (2012) observed significantly higher AST activity, without changes on ALT activity, in the blood plasma of dairy ewes fed probiotics compared with the controls. The AST activity may be due to increased metabolism of amino acids, as energy source or glucose precursors (Caldeira *et al.*, 1999), whereas increased cholesterol levels would indicate a greater absorption of nutrients in the intestines (Chiofalo *et al.*, 2004).

Reproductive performance and serum progesterone:

These results indicated that feeding lactating cows on diets supplemented with bacterial probiotics had beneficial effects on reproductive performance of lactating cows as compared control. It is of interest to note that dietary supplementation of bacterial probiotics markedly increased concentration of progesterone in blood serum of cows after 1st and 2nd insemination periods as compared to control group, but the differences were not significant (table 10). Result agreed with Mostafa *et al.* (2014) who reported that dietary supplementation of probiotics improved productive and reproductive performances of lactating cows. Such finding might be attributed either to stimulation of the immune system (Rodrigues *et al.*, 2000; Young, 2012) or improvement of the health condition of the supplemented cows by probiotics (Kalmus *et al.*, 2009).

Postpartum uterine involution:

The restoration of the uterus to its normal non-pregnant size and function after parturition is termed uterine involution which depends on the rate of

myometrial contractions, elimination of bacterial infection and the histological regeneration of the endometrium (Elmetwally *et al.*, 2016). Involution of the uterus is necessary before the cow can conceive again. As a result of the removal of the fetus, oxytocin and prostaglandin F_{2α} (PGF_{2α}) production reduced the uterine size (Edquist *et al.*, 1978). The reduction of uterine cervix and horn diameters early of dairy cows fed on probiotic supplemented diet compared to control reflected to better fertility and conception rate. Improvement of uterine involution may be to higher serum progesterone concentration in dairy cows fed on probiotic supplemented diet compared to control. The present data are supported by (Sheldon *et al.*, 2003) indicated that prostaglandins have a role in controlling uterine involution.

CONCLUSION

Using the produced probiotics supplement in dairy cows ration improved cows health and improved its transition period, BCS, milk production and milk quality. It also increased cows' energy utilization and improved reproductive performance and post-partum uterine involution. Also using probiotics generally did not adversely affect the levels of some biochemical indices of treated cows.

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تقييم تأثير استخدام محفزات نمو بكتيرية محلية الانتاج علي الكفاءة الانتاجية والتناسلية في أبقار الهولشتاين الحلابة

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تهدف هذه الدراسة لتقييم تأثير استخدام بكتيريا البروبيوتيك المنتجة محليا علي كفاءة الحالة الانتاجية والتناسلية للابقار الهولشتاين الحلابة هذه السلالات من البكتريا تم عزلها من اللعاب والروث في العجول الرضيعة ومن سائل الكرش في الابقار الحلابة. ثلاثون بقرة متعددة الولادات تم اختبارها اخرا ثلاث اسابيع من الحمل وتم تقسيمها الي مجموعتين (٥ لكل مجموعة) المجموعة الاولى كمجموعة ضابطة وغذيت علي عليقة انتظار ثم عليقة ما بعد الولادة بدون اضافة البروبيوتيك. المجموعة الثانية مجموعة المعاملة وغذيت علي نفس العليقة بالاضافة الي 10^6 CFU/kg الي كل كيلو جرام مادة جافة من البكتيريا المنتجة. تسجيل كمية اللبن لكل بقرة لمدة ثلاثة اشهر بعد الولادة. اظهرت النتائج ان الابقار المعاملة بالبروبيوتيك فقدت معدل اكتناز اقل مقارنة بالمجموعة الضابطة حتي ٣٠ يوم واكتسبت مجموعة المعاملة زيادة اكبر من المجموعة الضابطة عند ثلاثة اشهر بعد الولادة. اضافة البروبيوتيك لم يظهر زيادة معنوية في كمية المادة الجافة المأكولة خلال التجربة ولكن اضاقتة قامت بتحسين كمية اللبن بالنسبة لكمية الماكول. خلال التجربة ، لوحظ زيادة معنوية في الكولسترول وAST في مجموعة المعاملة اكبر من المجموعة الضابطة. لا توجد زيادة معنوية في كمية اللبن خلال التجربة ولكن كانت هناك زيادة بنسبة ٧.٣% في مجموعة المعاملة بينما لم تكن هناك زيادة معنوية في متوسط نسبة وكمية الدهن ونسبة المواد الصلبة غير الدهن ونسبة وكمية الرماد واللاكتوز . وجد ان البروبيوتيك له تأثير ايجابي علي الحالة التناسلية حيث ادى الي قصر الفترة المفتوحة وتقليل عدد التلقيحات وتقليل نسبة الشياخ المتكرر ومعدل اخصاب افضل بالنسبة للتلقيحة الثالثة ورجوع الرحم وعنق الرحم في مجموعة المعاملة عن المجموعة الضابطة. توصي هذه الدراسة استخدام البروبيوتيك الابقار الحلابة قام بتحسين معدل اكتناز الجسم في الفترة الانتقالية وزيادة كمية اللبن وجودته وله تأثير ايجابي علي الحالة التناسلية بعد الولادة.

الكلمات الدالة: بكتيريا البروبيوتيك ، كمية اللبن ، جودة اللبن والكفاءة التناسلية.