DOI: 10.17311/sciintl.2014.13.19

Influence of Propionibacteria Supplementation to Rations on Intake, Milk Yield, Composition and Plasma Metabolites of Lactating Buffalos During Early Lactation

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ABSTRACT

Background: A probiotics (direct-fed microbials, DFM) and prebiotics (microbial growth promoters) are specific agents may have provide alternatives to chemical modifiers of manipulate the microbial ecosystem and fermentation characteristics in the rumen and intestinal tracts of livestock animals. The objective of this study was evaluating the effect of two levels propionibacteria strain (P169) on buffalo's performance. **Methods:** Six lactating buffaloes in early lactation after four days from calving were divided to three treatments by using two blocks 3×3 complete switch–back design and three successive experimental periods. Each period consisted of 30 days. Animals were fed rations consisting of 70% Concentrate Feed Mixture (CFM) and 30% roughage [20% berseem (B) and 10.0% Rice Straw (RS)] on dry matter basis as control (1). Dietary treatments were: Control ration plus 2 g dairy ProP169® (low dose) (*Propionibacterium freudenreichii* strain P169) (2) and control ration plus 4 g dairy ProP169® (high dose)/head/day (3). **Results:** Buffaloes fed supplemental Propionibacteria had lower DMI kg^{-1} b.wt. ($p < 0.05$) than control. Body weight was improved by treated P169 ($p < 0.05$). But, treatments did not affect nutrients digestabilities. Milk yield and components were not significantly ($p > 0.05$) differed between all treatments. Also, blood plasma parameters were not affected by treatments groups. **Conclusion:** It could be concluded that supplementing rations with dairy Prop P169 might has a little effect on lactating buffalo's performance.

Key words: Propionibacteria, digestibility, milk yield, milk composition, lactating buffaloes

Science International 2 (1): 13-19, 2014

INTRODUCTION

Direct fed Microbials (DFM) are defined as a source of live, naturally occurring microorganisms[1]. They are utilized in dairy production to improve animal performance, feed efficiency and health[2]. Ruminally derived propionate is the major precursor for gluconeogenesis in early lactation dairy cows[3] and increasing ruminal synthesis of propionate may increase glucose supply, reduce ketosis and provide increased substrate for lactose synthesis. Moreover, the efficiency of utilization for maintenance of propionic acid is 0.86 versus 0.59 for acetate and 0.76 for butyrate[4] and thus directly feeding propionibacteria may be a natural way to increase hepatic glucose production and positively influence metabolism[5]. There are many bacterial based DFM that are sold for use in ruminant diets with more specific applications. One of the most common microorganisms used is propionibacteria.

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Propionibacteria may be beneficial when fed to ruminants. These bacteria are naturally found in high numbers in the rumen of animals fed forage and medium concentrate diets that make up 1.4% of ruminal microflora and produce propionate, a major precursor for glucose production by hepatic gluconeogenesis[6,7]. Propionibacteria have the ability to convert lactic acid and glucose to acetic and propionic acid, therefore they are probably too slow growing and acid intolerant to prevent an acute lactic acidosis challenge. It was observed that, a definitive mode of action for bacterial or fungal DFM has not been established, although a variety of mechanisms have been suggested. These include the modification of rumen or lower gut microbial populations, alteration of rumen fermentation patterns, increased intestinal nutrient flow, improved diet digestibility and immune system modulation[1,2]. Currently available data on effects of propionibacteria on dairy cows are limited and inconsistent from where dose additives and concentrate diets level. The objective of this study was to determine the effect of feeding (2 levels) a

bacterial DFM containing *P. freudenreichii* strain P169 with high concentrate diets on milk yield and composition, milk fatty acids, nutrient digestibility and blood plasma metabolites of lactating buffaloes in early lactation.

MATERIALS AND METHODS

This experiment was conducted at the Cattle Information Systems/Egypt (CISE), Faculty of Agriculture, Cairo University, Egypt and Dairy Science Department, National Research Center, Egypt. During winter season.

Animals and treatments: Six multiparous lactating buffaloes at second and third season of lactation, with an average body weight of 551 kg were used in the present study. Buffaloes were randomly divided after 4 days of calving into two blocks 3x3 switchback complete design[8]. The experimental period was 90 days for three periods; each period consisted of 21 days for adaptation and 7 days as collection period for samples of milk, feces and blood. Lactating buffaloes were individually fed to cover requirements according to Ghoneim[9]. Rations were recalculated every two weeks based on milk yield and body weight of animals. Buffaloes in each group were fed rations consisting of 70% Concentrate Feed Mixture (CFM) and 30% roughage (20% berseem (B) (*Trifolium alexandrinum*) and 10.0% Rice Straw (RS) on Dry Matter (DM) basis, approximately. Dietary treatments were (1) Control group were received this ration without supplement; (2) Control ration plus 6×10^{10}CFU/2 g dairy ProP169® (low dose) (*Propionibacterium freudenreichii* strain P169) and (3) Control ration plus 1.2×10^{11}CFU/4 g dairy ProP169® (high dose)/head/day. The *propionibacteria* strain 169 (dairy ProP169®) as direct fed microbial for dairy cattle is a dried material of viable bacteria prepared by Bio-vet Inc. USA. Chemical analysis of dietary ingredients is present in Table 1. Proximate analysis of dietary ingredients and nutritive value of experimental diets are presented in Table 2. Concentrate feed mixture was offered two times daily after at 8.00 am and at 6.00 pm, berseem once daily at 11 am and rice straw was given once daily at 2 pm. The additives were offered for buffaloes on top dressed once a day in the morning feeding. Buffaloes were allowed to drink water after each meal and overnight and were kept under the routine veterinary supervision throughout the whole feeding trial.

Sampling collection and analysis: Buffaloes were weighed at the start of the experiment, when moved to the tie stalls (4 days in milk, DIM) and then every 2 week. Feed offered and refused was measured in last 3 days from each period to calculate Dry Matter Intake (DMI). Samples of tested feedstuffs were taken at the beginning, middle and end of the collection period. Feces samples were taken from the rectum of each animal twice daily at 10 am and 5 pm for three successive days from each animal during the collection period. The proximate analysis for feeds and feces were analyzed according to AOAC[10]. Crude Fiber (CF), Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) were analyzed by ankom200 fiber analyzer (Ankom Technology Corporation, Fairport, NY). Acid Detergent Lignin (ADL) Acid detergent lignin was determined by digesting the ADF residue in 72% sulfuric acid[11]. Dry matter and nutrient digestibilities were determined according to the acid insoluble ash technique (AIA)[12]. Live bacterial numbers of product

Table 1: Chemical composition of concentrate feed mixture (CFM), Berseem (B) and rice straw (RS) (on dry matter basis)

	Diet ingredients		
Items	CFM*	B	RS
Dry matter	86.85	15.95	91.86
Organic matter	91.10	86.00	86.03
Crude protein	15.79	17.69	04.04
Ether extract	03.98	03.52	02.35
Nitrogen free extract	64.83	39.71	45.85
Crude fiber	06.50	25.08	33.79
Ash	08.90	14.00	13.97
Cell wall constituents			
NDF	25.73	50.85	73.83
ADF	11.44	37.18	53.08
ADL	02.62	11.48	13.98
Hemicelluloses	14.29	25.70	20.75
Cellulose	08.82	23.47	39.10

*CFM consisted of 25% undecorticated cotton seed meal, 35% wheat bran, 30% corn, 3% rice bran, 3% molasses, 2% limestone, 1% urea and 1% salt (NaCl). NDF: Neutral detergent fiber, ADF: Acid detergent fiber, ADL: Acid detergent lignin

Table 2: Dietary ingredients composition of the experimental rations and chemical analysis (on dry matter basis)

Items	Control	P169 (L)[1]	P169 (H)[2]
Composition (%)			
Concentrate feed mixture	66.81	70.70	71.54
Berseem	22.97	18.34	17.37
Rice straw	10.22	10.96	11.09
Pro P169®	-	0.20	0.40
Chemical composition (%) (calculated)			
Dry matter	71.07	74.39	75.09
Organic matter	89.41	89.61	89.65
Crude protein	15.03	14.85	14.82
Ether extract	3.71	03.72	03.72
Crude fiber	13.56	12.90	12.75
Nitrogen-free-extract	57.12	58.14	58.36
Ash	10.59	10.39	10.35
Cell wall constituents (calculated)			
NDF	36.42	35.61	35.43
ADF	21.61	20.72	20.53
ADL	05.82	05.50	05.42
Hemicelluloses	14.82	14.89	14.90
Cellulose	15.27	14.82	14.72

[1]P169 (L): Propionibacteria P169 (low dose), [2]P169 (H): Propionibacteria, P169 (high dose), NDF: Neutral detergent fiber, ADF: Acid detergent fiber, ADL: Acid detergent lignin

was enumerated with specie specificity for genus *P. freudenreichii*. DFM package was analyzed for total counts of propionibacteria. All samples were analyzed in duplicate. Samples (1 g) were transferred to tubes containing buffered saline water and serially diluted to 10^9 and 1 mL aliquots of this dilution were plated onto Sodium Lactate Agar (SLA) plates[13]. SLA plates were incubated anaerobically in a sealed jar under an O_2-free CO_2 atmosphere at 37°C for 7 days. After incubation, colonies were counted and the live bacterial count was calculated using the dilution factors. Colonies on SLA were identified with genus specificity as Propionibacteria with analytical profile index strips specific for anaerobic organisms. During lactation, buffaloes were machine milked twice daily and milk weights were recorded. During the last 4 days of each period, milk samples from consecutive evening and morning milking were mixed in proportion to yield. Milk constituents of total solids, fat, protein, lactose, solids non fat and ash were analyzed by Bentley[150] infrared milk analyzer (Bentley Instruments, Chaska, MN, USA) calibrated for buffaloes milk analysis on individual buffaloes pooled evening and morning milk samples on a proportional volume basis. Fatty acids profile of milk fat was determined as methylated according to Park *et al.*[14] and separated by gas liquid chromatography. Blood samples were collected at the final day of the treatment period at four hours after morning concentrate feeding at 8:00 am and water from the jugular vein. Blood samples were collected into clean dried glass culture tubes containing EDTA as anti-coagulant and centrifuged at 4000 rpm for 20 min; blood plasma was then transferred into a clean dried glass vial and then stored at -18°C till chemical analysis. Commercial test kits were used for measurement of plasma glucose according to Trinder,[15] and Young*et al.*[16], triglycerides[17] (Stanbio Laboratory, Boerne, TX), cholesterol[18,19], $-Hydroxybutyric acid, $-HBA (BEN Biochemical Enterprise, BEN S.r.l., Milan, Italy)[20,21] and plasma total lipids (Biodiagnostic, Cairo, Egypt)[22]. The biochemical analyses of the selected parameters were spectrophotometrically measured (T80 UV/VIS Spectrometer, PG Instruments Ltd, UK) according to the standard protocols of the suppliers.

Statistical analysis: The data were subjected to statistical analysis according to Lucas[8]. Significance between the means was determined by multiple range test[23].

RESULTS AND DISCUSSION

Body weight, Feed intake and nutrients digestibility: Average Body Weight (BW) of lactating buffaloes were insignificantly ($p > 0.05$) differed among treatments as shown in Table 3. The body weight was increased with high dose and low dose supplemented diets compared to control diets, respectively. There were not significant differences among treatments detected in the overall means of Dry Matter Intake (DMI) or Digestible Crud Protein (DCP) and all nutrients digestibility. However, Total Digestible Nutrients (TDN) were higher ($p < 0.05$) with the control group than experimental additives (Table 3). In this study have not DMI is affected by the treatments. This is because the propionate receptors in the ruminal region might function to control feed intake, propionate is a stimulant of insulin secretion in cattle which may indirectly influence appetite[24]. Also, Ghorbani *et al.*[25] using P169 in rations of dairy animals, they reported that dry matter intake was not affected by the experimental additives. Similar results were obtained by Raeth-Knight *et al.*[26]; Thompson[27] and West and Bernard[28]. In contrast, De Ondarza and Seymour[29] reported supplementation of P169 increased DMI. Supplementation of P169 was not significantly affected on all nutritive value. This result agrees with Raeth-Knight *et al.*[26] who, found no effects on apparent digestibility of DM, CP and NDF with feeding of combination of *L. acidophilus* LA747, *L. acidophilus strain* LA45 and *P. freudenreichii* PF24 on dairy cattle performance. Akay and Dado[30] concluded that addition of *Propionibacterium* strain P5 reduced DM and NDF digestibility of feeds *in vitro* by 2 and 14%, respectively compared to control. On contrast, Lehloenya *et al.*[31] reported that addition of *Propionibacterium* strain P169

Table 3: Effect of propionibacteria (P169) supplement on body weight (BW), dry matter intake (DMI), Total digestible nutrients (TDN) intake, Digestible crude protein (DCP) intake, nutrients digestibility and nutritive value

	Treatments			
Items	Control	P169 (L)[1]	P169 (H)[2]	±SE
BW (kg)	549.9	552.50	551.50	04.55
DMI (kg hG[1] dayG[1])	14.27	13.14	13.35	00.32
TDN intake (kg hG[1] dayG[1])	8.91[a]	08.20[b]	08.07[b]	00.19
DCP intake (g hG[1] dayG[1])	1299.3	1293.80	1241.90	34.79
Nutrients digestibility (%)				
Dry matter	61.75	61.22	62.04	00.91
Organic matter	65.02	65.65	63.35	01.81
Crude protein	63.17	66.39	62.77	01.45
Crude fiber	53.74	54.85	53.82	01.58
Ether extract	62.43	63.17	60.48	00.85
Nitrogen-free-extract	68.61	67.51	65.50	03.21
NDF	55.97	55.90	55.70	00.72
ADF	51.98	53.42	52.32	00.65
Nutritive value (%)				
DCP	9.200	09.86	09.32	0.23
TDN	63.26	62.61	60.38	1.87
NE_l, Mcal kgG[3]	1.430	01.42	01.36	0.05

[1]P169 (L): Propionibacteria P169 (low dose); [2]P169 (H): Propionibacteria P169 (high dose), [3]NE_l: Net energy lactation was estimated by equation according to (NRC, 2001). NDF: Neutral detergent fiber, ADF: Acid detergent fiber. Means with different superscripts in the same row are significant ($p < 0.05$)

Table 4: Effect of propionibacteria (P169) supplement on plasma blood metabolites

Items	Treatments			
	Control	P169 (L)[1]	P169 (H)[2]	±SE
Glucose (mg dL^{-1})	65.30	71.80	076.92	04.02
Cholesterol (mg dL^{-1})	142.70	155.48	171.06	19.69
$-HBA (mmol L^{-1})	1.12	00.75	000.90	00.22
Total lipid (mg dL^{-1})	428.60	469.30	476.90	53.96
Triglyceride (mg dL^{-1})	101.60	102.80	107.80	11.91

[1]P169 (L): Propionibacteria P169 (low dose), [2]P169 (H): Propionibacteria P169 (high dose)

improved of DM, NDF and ADF digestabilities vs. control diet. Boyd[32] demonstrated a significant effect on the apparent digestibility of DM, CP, ADF and NDF for treatments with supplementations versus control. The addition of propionibacteria resulted in lower DM and NFE digestibility when compared with control. Robinson *et al*.[33] proposed that a decrease in NDF digestion may have occurred because of increased Volatile Fatty Acids (VFA) concentrations, particularly acetate which may inhibit acetate producing bacteria, thus slowing the rate of fiber digestion.

Blood plasma parameters: Feeding with P169 supplementation on plasma glucose, cholesterol, $-Hydroxybutyrric acid ($-HBA), total lipids and triglyceride concentrations of lactating buffaloes were no significant differences are present in (Table 4). However, there were tendency for linear increase plasma glucose (5-14% greater) and lipids metabolites for treated groups than control while plasma $-HBA concentrations insignificantly ($p > 0.05$) lower for buffaloes fed the low and high dose P169 compared with control. This result is agreed with reported by Francisco *et al*.[5] and Klebaniuk *et al*.[34]. Van Knegsel *et al*.[35] concluded that the glucogenic diet was effective in improving the calculated energy balance and decreasing plasma $-hydroxybutyrate and they suggested that a reduced risk of metabolic disorders in multiparous dairy cows fed a glucogenic diet. Lean *et al*.[36] found that, in multiparous cows, glucose concentrations are negatively cross correlated with $-hydroxybutyrate concentrations and the estimated net energy balance is negatively cross correlated with $-hydroxybutyrate. Some authors suggested that, the level of triglycerides constitute the major form of fat reserve stored in the organism and secondary substrate for gluconeogenesis in ruminants and released to the circulation when needed[37,38], contribution to glucose production has been estimated to be small amounts (about 3-4%) for glycerol[39]. These results confirm the data of Lehloenya *et al*.[31] and Boyd[32], who observed that plasma glucose concentration was not affected with propionibacteria supplementation. In the same context, Thompson[27] found that there no statistical differences

Table 5: Effect of propionibacteria (P169) supplement on milk yield and composition

Items	Treatments			
	Control	P169 (L)[1]	P169 (H)[2]	±SE
Milk (kg day^{-1})	9.96	10.50	10.45	0.34
4% FCM[3]	15.85	16.25	16.57	0.58
Milk composition (%)				
Fat	7.99	7.69	7.94	0.18
Total solids	18.01	18.04	18.35	0.23
Solids-not-fat	10.02	10.35	10.31	0.18
Total protein	3.84	3.87	3.89	0.02
Lactose	5.15	5.13	5.14	0.05
Milk constituent yield (kg day^{-1})				
Fat yield	0.791	0.803	0.826	0.03
Total solids	1.79	1.89	1.91	0.06
Solids-not-fat	1.00	1.08	1.07	0.03
Total protein	0.380	0.404	0.403	0.01
Lactose	0.513	0.539	0.538	0.02

[1]P169 (L): Propionibacteria P169 (low dose), [2]P169 (H): Propionibacteria P169 (high dose), [3]FCM: Fat corrected milk was calculated by the following equation according to Gains[42]. FCM = 0.4 M + 15 F, where, M = Milk yield (kg d^{-1}), F = An amount of fat = M × fat %

were observed among treatments on glucose and $-hydroxybutyrate when used of 10^9 CFU g^{-1} of *Lactobacillus acidophilus* and 10^9 CFU g^{-1} *Propionibacterium freudenreichii* (DFM) 4 Holstein cows equipped with ruminal cannulas. However, Aleman *et al*.[40] found that plasma glucose was increased ($p < 0.05$) by propionibacteria supplementation.

Milk yield and composition: Milk yield, 4% Fat-corrected Milk (FCM) were insignificantly ($p > 0.05$) slightly increased in lactating buffaloes in response to dietary P169 supplementation compared with control ration to being (100, 105.4 and 104.9%) for the control, low dose, high dose supplemented diets, respectively (Table 5). No significant differences for milk composition percentages and yields. However, milk constituent yields were higher ($p > 0.05$) insignificant among dietary treatment groups vs. control (Table 5). Similar results were reported by Raeth-Knight *et al*.[26]. The nonsignificant effects of propionibacteria on milk yield and composition may be due to the incompatibility of buffalo with these additions. These results also are agreed with that noted by Thompson[27], who supplemented of 10^9 CFU g^{-1} of *Lactobacillus acidophilus* and 10^9 CFU g^{-1} *Propionibacterium freudenreichii* as the DFM for dairy cows and had no difference effects on milk production and milk components (fat, protein, lactose, SNF%) compared to control cows. However, the positive effect for using DFM as propionibacteria was detectable in several studies, Stein *et al*.[41] reported that early lactation, multiparous cows offered 6×10^{10} or 6×10^{11} CFU/day of *Propionibacterium* strain P169 produced about 7.1 and 8.5% increases above controls in daily 4% fat corrected milk for high dose and low dose P169 treated cows,

Table 6: Effect of propionibacteria (P169) supplement on milk fatty acids composition

Items	Treatments			
	Control	P169 (L)[1]	P169 (H)[2]	±SE
$C_{6:0}$	00.75	01.16	00.63	0.77
$C_{8:0}$	00.89	01.07	00.85	0.47
$C_{10:0}$	01.99	02.12	02.46	0.27
$C_{12:0}$	02.92	02.92	03.44	0.16
$C_{14:0}$	14.10	14.08	15.05	0.84
$C_{14:1}$	00.49	00.67	00.63	0.07
$C_{15:0}$	01.14	01.10	01.18	0.24
$C_{16:0}$	37.48	38.26	38.00	1.06
$C_{16:1}$	01.39	01.65	01.46	0.39
$C_{17:0}$	00.35	00.64	00.55	0.14
$C_{18:0}$	13.00	11.00	11.19	1.37
$C_{18:1n9c}$	20.62	20.38	20.00	2.13
$C_{18:1n9t}$	01.69	02.31	02.23	0.30
$C_{18:2n6t}$	01.14	00.35	00.52	0.53
$C_{18:2n6c}$	00.56	00.69	00.42	0.26
$C_{18:3n6}$	00.70	00.76	00.68	0.44
$C_{18:3n3}$	00.11	00.23	00.19	0.04
$C_{20:0}$	00.57	00.50	00.37	0.37
$C_{20:1}$	00.10	00.12	00.15	0.04
Total saturated (S)	72.79	72.67	73.80	4.11
Total unsaturated (U)	27.21	27.33	26.20	4.11
Monosaturated	27.79	29.26	28.08	9.44
Diunsaturated	01.66	01.03	00.94	0.75
Polyunsaturated	00.80	00.99	00.88	0.25
U/S ratio	00.38	00.38	00.36	0.001

[1]P169 (L): Propionibacteria P169 (low dose), [2]P169 (H): Propionibacteria P169 (high dose)

respectively. Moreover, De Ondarza and Seymour[29] reported that milk production was positively affected ($p = 0.04$) by propionibacteria supplementation during of early lactation period with overall milk production was excellent throughout the study with means of 44.31 and 43.06 kg day^{-1} for the propionibacteria supplemented and control diets, respectively. Also, West and Bernard[28] observed that supplemented two bacterial inoculants (*Propionibacterium freudenreichii* strain NP24, *Lactobacillus acidophilus* strain NP51 and *L. acidophilus* strain NP45) to lactating holstein cows had greater yields of milk fat, FCM and energy corrected milk than did controls.

Milk fatty acids profile: Effects of *propionibacteria* P169 supplementation on milk fatty acids composition of lactating buffaloes were not ($p > 0.05$) significantly differ among treated groups and control are shown in (Table 6). Generally, there were ($p > 0.05$) improved for some milk fatty acids determined either with low-dose or high-dose P169 compared with control. Similar results were reported by Thompson[27] who found that no significant effect of DFM on total milk fatty acid classes (saturated fatty acids, unsaturated fatty acids, monosaturated fatty acids, polyunsaturated fatty acids, n-6 polyunsaturated fatty acids, n-3 polyunsaturated fatty acids and conjugated linoleic acid).

CONCLUSION

Supplementation of propionibacteria P169 at low dose level in rations of lactating buffalo's diets during early lactation affected negatively on DMI and nutrients intake but improved body weight. Milk yield, milk components and FCM were insignificantly increased with P169 group vs. control.

ACKNOWLEDGMENTS

The authors express appreciation to Bio-Vet, Inc (Blue Mounds, WI), especially of gratefully acknowledge for Mr. Bill Helwig, Marketing Director of Bio-Vet, Inc for helping and for providing the bacterial inoculants for carrying out this research.

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