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Impact of Dietary Propylene Glycol Supplementation on Some Biochemical Parameters, Productive and Reproductive Performance of Lactating Dairy Cows

Eldsokey Nassef [1*], Tarek K. Abouzed[2], Mustafa Shokry[3]
[1] Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Kafrelsheikh University, 33516, Egypt
[2] Department of Biochemistry, Faculty of Veterinary Medicine, Kafrelsheikh University, 33516, Egypt
[3] Department of Physiology, Faculty of Veterinary Medicine, Kafrelsheikh University, 33516, Egypt

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***Corresponding to:**
dsokeynassef@yahoo.com

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ABSTRACT

The aim of this study was to determine the effect of propylene glycol on milk yield, milk composition, and parameters of subclinical ketosis and reproductive performance of high producing dairy cows. Twenty Holstein cows in the close up period were distributed into two equal groups. The control group received the basal diet. The other group was supplemented with propylene glycol (PG) (300 g per cow per day). The dietary treatment lasted from 3 weeks prepartum to 8 weeks postpartum. Body condition scores (BCS) were monitored using 1 to 5 scale at the start, parturition, 4 weeks and 8 weeks postpartum. All the cows were kept in tie-stall barns. Milk yield was daily recorded for each cow. Milk samples were collected weekly and analyzed for composition and somatic cell count (SCC). Blood samples were collected from all cows on day 10 prepartum to estimate non- esterified free fatty acids (NEFA). Further blood samples were collected on day 30 postpartum to determine serum NEFA, beta hydroxyl butyric acid (BHBA), urea, triglycerides and liver enzymes. Also, on day 30 postpartum, milk samples were collected to measure BHBA. Conception rate, days open and service pre conception were recorded for all cows. Cows supplemented with PG significantly had better BCS (3.2±0.06) than the control cows (2.8±0.04) at 8 weeks post-partum. PG significantly increased milk yield by 32.26% and milk protein by 32.9% than the control, while the other milk constituents and SCC were nearly similar in the two groups. Also, PG improved the energy status of the cows through increasing blood glucose level (57.8 ±1.1 versus 30±3.1) mg/dl and decreasing prepartum NEFA (0.3±0.02 versus 0.42±0.04) mmol/l. Also, lowering post-partum BHBA (0.73±0.12 versus 1.16±0.23) mmol/l. Moreover, PG significantly improved the reproductive performance of the lactating cows through shortening of days open by 31.0%, and decreasing service per conception by 23.08%. Finally, practical dietary supplementation of PG could be used to increase milk yield, improve reproductive performance and prevent subclinical ketosis of Holstein cows.

1. INTRODUCTION

The modern Holstein cows have the high genetic potential for milk yield which possesses them at high risk for metabolic diseases such as ketosis and fatty liver. Additionally, improper nutrition and management always associated with these diseases (Oetzel, 2004). Severe economic losses in dairy farms resulted from ketosis and fatty liver. These losses are due to lowering milk yield, affecting reproductive performance and increasing culling rates (McArt et al., 2015).

Dairy cows experience a linear increase in milk yield after parturition till reaching the peak of milk yield at 1 to 2 months of lactation and persist for nearly 5 months postpartum Ruelle et al., (2019). Moreover, during this period, the dairy cows experience low dry matter intake (DMI) because of hormonal changes and diet exchange from close up to lactating diet. Dairy cows depend on body store of protein and triglycerides to meet the nutrients requirement for lactation Krumm et al., (2017). So, dairy cows physiologically experience loss of body

condition score (BCS) during early lactation and suffer from negative energy balance (NEB). The role of nutrition is to minimize NEB and to avoid greater loss of body weight by encouraging DMI, animal welfare and use of energy supplement.

Indicators of Lipid mobilization in ruminants are BHBA and NEFA (Gonzales *et al.,* 2011). Excessive blood NEFA is greatly accumulated as triglycerides (TG) in the liver, resulting in fatty liver (Sevinc *et al*. 2003). Fat deposition in the hepatocytes compresses cell organelles. That is leading to hepatocyte degeneration and releases of cytoplasm enzymes (Jóźwik *et al.,* 2012). Blood BHBA concentration of 1.2 mmol/L has often been used for the detection of animals with subclinical ketosis (Duffield *et al*., 1997). However, blood sampling possesses stress factors on the animals. The Determination of ketone bodies in milk can make sampling easier. Moreover, semi-quantitative cow-side tests for determination of milk BHBA) recently became available and can provide an immediate result. The literature does not provide extensive data for comparing concentrations of ketone bodies in milk and blood.

Propylene glycol (PG) is converted to pyruvate, which eventually converted to glucose (Van Soest, 1994). Gordon et al., (2017) concluded that PG could increase milk production during early lactation. Also, they suggested more research is required to understand the relation between blood glucose and BHBA. Blood glucose provides the oxaloacetate that is essential for complete oxidation of fatty acids in mitochondria giving greater energy. Otherwise, fatty acids would incompletely be oxidized resulting in the production of ketone bodies and lower energy (Nielsen and Ingvartsen, 2004). Therefore, this study aimed to investigate the addition of dietary PG from 3 weeks prepartum to 8 weeks postpartum on milk yield, milk composition, parameters of subclinical ketosis and reproductive performance in multiparous Holstein cows.

2. MATERIALS AND METHODS

2.1. Animals and diets

Three weeks prepartum, 20 multiparous Holstein cows were allocated into two equal groups. The first group was the control one which received the basal diet (Table 1). The other group was supplemented with PG (300 g per cow per day). The dietary treatment lasted from 3 weeks prepartum to 8 weeks postpartum. Body condition scores (BCS) were recorded using the 1 to 5 scale according to Ferguson et al., (1994), with 1 meaning = too thin and 5 =too fatty. All the cows were maintained in tie-stall barns. The chemical analysis of the diets (Table 2) was performed according to AOAC (2010). Total digestible nutrients (TDN) were calculated by equations of NRC (2001).

The PG was firstly mixed with the concentrate portion of the diet then added to the total mixed ration of the experimental cows. During close up period the chloride level was increased to make compensatory acidosis in order to prevent hypocalcemia. The compensatory acidosis increased renal calcium excretion. So calcium level should be increased during acidification of the diet.

Table 1. Ingredients composition of close up and early lactating diets on dry matter basis

Ingredients ٪	**Close up diet**	**Early lactating diet**
Alfalfa hay	8.71	19.29
Corn silage	46.35	26.47
Corn grains	18.41	25.39
Soybean meal	11.88	19.33
Wheat bran	7.51	4.02
Sugar beet bulb	3.68	1.03
Calcium chloride	0.98	-
Salt	0.33	0.27
Dried fat (ca soaps of FA) [a]	0.38	1.50
Yeast (XPC, Diamond V)	0.07	0.07
Acid buf [b]	0.40	0.40
Anti-mycotoxin	0.13	0.13
Limestone	0.38	1.60
Magnesium oxide	0.49	0.20
*Premix	0.30	0.30

*Premix provides 5500 IU vitamin A; 500 IU vitamin D3; 50 IU vitamin E; 60 mg iron; 80 mg zinc; 40 mg manganese; 20 mg copper; 0.6 mg iodine; 0.3 mg selenium and 0.25 mg cobalt per 1kg dry matter of the diet. [a] Dried fat contained dry matter 95.5%, Ash 12%, crude fat 84%, and calcium 9%. [b] Acid buf contained ash 96%, Calcium 30%, magnesium 5%, sodium 1.2%.

Table 2. Nutrients composition of close up and early lactating diets on dry matter basis

Nutrient ٪	Close up diet	Early lactating diet
Crude protein	13.90	16.60
Ether extract	2.87	4.01
Neutral detergent fiber	36.40	29.60
Forage NDF	33.40	20.90
Acid detergent fiber	24.70	18.10
Nonfiber carbohydrate	37.60	43.50
Total digestible nutrients	64.00	72.00
Calcium	1.20	0.80
Phosphorous	0.30	0.40

Total digestible nutrients were calculated according to NRC, 2001.

2.2. Sampling and laboratory analyses

Subsamples of close up and lactating diets were ground to pass 1 mm screen (Cyclotec 1093, Foss Sweden). The ground samples were analyzed for dry matter (105 °C for 3h in air-forced oven; method 934.01), ash (600 °C in muffle furnace for 2 h; method 942.05), ether extract (Soxhlet procedure; method 2003.05) and crude protein (Kjeldahl procedure; method 2001.11). The fiber fractions were determined using the ANKOM2000 fiber analyzer apparatus (ANKOM Technology Cooperation, Fairport, NY, USA). Neutral detergent fiber analysis was performed using sodium sulfite and alpha amylase (heat stable) and expressed with residual ash content. Non-fibrous carbohydrates (NFC) content was estimated using the formula: NFC = DM − (CP + EE + ash + NDF).

Blood samples were collected from all cows on day 10 prepartum for NEFA estimation. Further blood samples were collected on day 30 postpartum for serum NEFA, BHBA, glucose, urea, triglycerides, AST, and ALT. At the same time, Milk samples were collected to measure BHBA. Further milk samples were collected weekly for analyses of total solids, protein, lactose, fat and somatic cell count. For each cow, a coccygeal blood sample was taken before the afternoon milking into 10-ml glass tubes. These tubes were refrigerated at 4°C in the ice pox, and within 2 h were centrifuged at 3000 rpm for10 min. The collected sera were frozen at−20°C until analyses.

Blood glucose, triglycerides, and urea nitrogen were measured by colorimetric methods according to Tietz, (1995). Serum enzyme activities of AST and ALT were measured according to Bergmeyer et al., (1976) using kits from Randox) Ireland). Serum NEFA concentration was measured according to Murray et al., (1984) using diagnostic reagent for quantitative determination on photometric systems. The concentration of serum BHBA was determined according to Burtis and Tietz, (1999) using quantitative determination.

About 100 ml of milk was sampled during the beginning of milking. A quantitative determination of milk BHBA concentration with the Ketolac strip was performed immediately. Results were denoted according to the milk BHBA concentration (mmol/L).

Milk yield was recorded daily for each cow for 8 weeks. Milk samples were collected weekly for chemical analyses of total solids, lactose, protein and somatic cell count using a milk scan (Foss, Sweden).

2.3. Statistical analyses

All the data were analyzed using SPSS statistical analysis software (version 16). The data were analyzed by the T-independent samples T-test. Differences detected at $P<0.05$ using Levene's Test were considered statistically significant.

3. RESULTS

At the start of the experiment, the BCS of both groups were nearly similar (Table 3). Also, there was no significant difference between the two groups at parturition and at 4 weeks post-partum. The significant difference in BCS was detected at 8 weeks postpartum where the cows fed PG had a higher body condition score than those in the control group.

Milk yield of cows supplemented with PG was dramatically higher than that of the cows in the control group (Table 4). PG increased milk yield by 32.26% more than the control group. With regard to milk composition (Table 4), there were no significant differences in milk fat, lactose, total solids and solids not fat between the two groups. On the other hand, cows supplemented with PG had a significantly higher milk protein % (3.07±0.14) than those in the control group (2.31 ±0.17).

The results of serum biochemical parameters are shown in Table 6. Cows supplemented with PG had significantly higher levels of glucose and triglycerides than the control cows. In contrast, the blood urea level was significantly lower in cows supplemented with PG than the control cows.

There were no significant differences in the activities of AST and ALT enzymes among the two groups.

The results of the parameters of ketosis are shown in Table 5. Cows supplemented with PG had significantly lower serum NEFA prepartum. While there was no significant difference in serum NEFA among the two groups postpartum. Whereas, BHBA in both blood and milk was significantly lower in cows supplemented with PG than the control cows.

Results of dietary PG on the reproductive performance of Holstein cows are shown in Table 7. Cows supplemented with PG displayed the first estrus more rapidly than the cows of the control one. Also, PG significantly decreased service per conception by 23.1% and shortened days open by 31.2% as compared to the control.

Table 3. Impact of dietary propylene glycol on body condition score of Holstein cows during transition and lactation periods

Body condition score	Control	Propylene glycol
3 weeks pre-partum[1]	3.70±0.10	3.65±0.16
At parturition	3.63±0.10	3.58±0.10
4 weeks postpartum	3.35±0.05	3.10±0.10
8 weeks post-partum	3.20±0.06[a]	2.80±0.04[b]

Means±SE with different letters are significantly different (P<0.05)

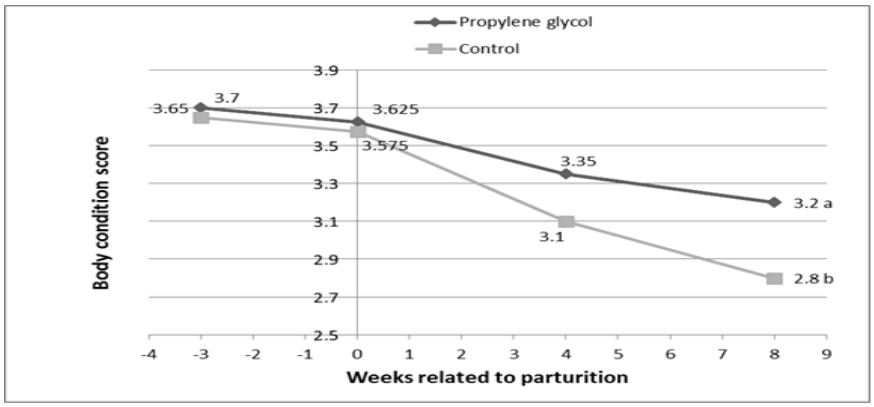

Figure 1. Impact of dietary propylene glycol (300 g/cow/day) on body condition score o multiparous Holstein cows

Table 4: Impact of dietary propylene glycol on milk yield and milk composition of Holstein cows

Item	Control	Propylene glycol
Milk yield (kg/day)	34.90 [b] ±1.35	41.00 [a] ±1.35
Fat %	4.40±0.73	4.05±0.36
Protein %	2.31 [b] ±0.17	3.07 [a] ±0.14
Lactose %	3.35±0.34	3.67±0.24
Total solids %	10.90±1.04	11.48±0.15
Solids, not fat %	6.50±0.39	7.43±0.38
Somatic cell count no/ml	722250±412	680750±347

Means±SE in the same row with different letters are significantly different P<0.05.

Table 5: Impact of dietary propylene glycol on ketosis parameters of Holstein cows

Item	Control	Propylene glycol
Pre-partum NEFA**(mmol/l)**	0.42±0.04 [a]	0.3±0.02 [b]
Post-partum NEFA **(mmol/l)**	0.41±0.07	0.34±0.03
Blood BHBA **(mmol/l)**	1.16±0.23 [a]	0.73±0.12 [b]
Milk BHBA **(mmol/l)**	220±34 [a]	115±15 [b]

Means±SE in the same row with different letters are significantly different (P<0.05). Pre-partum NEFA, non-esterified fatty acids measured 10 days before parturition; Post-partum NEFA measured 30 days after parturition; BHBA, beta hydroxyl butyric acid measured in blood and milk at 30 days after parturition.

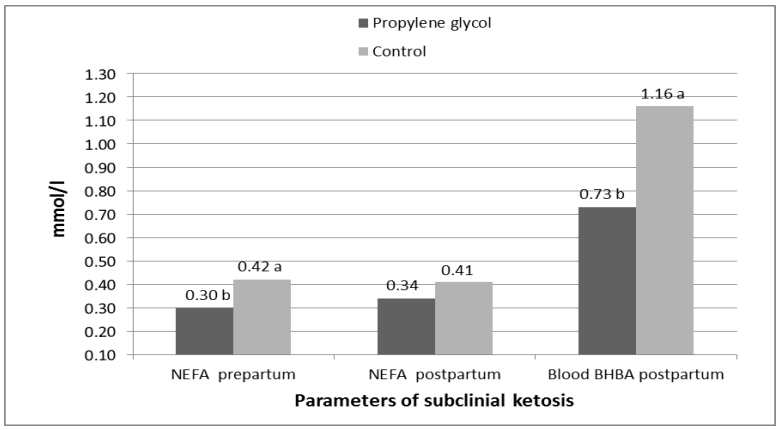

Figure 2. Impact of dietary propylene glycol (300 g/cow/day) on parameters of subclinical ketosis of Holstein cows

Table 6: Impact of dietary propylene glycol on serum biochemical parameters of lactating Holstein cows

Items	Control	Propylene glycol
Glucose (mg/dl)	30.00±3.1 [a]	57.80 ±1.10 [b]
Urea (mg/dl)	35.5±0.27 [a]	28.55±0.31 [b]
Triglycerides (mg/dl)	84±15 [b]	137±11 [a]
Aspartate aminotransferase (U/I)	51.00±5.10	42.70±6.50
Alanine aminotransferase (U/I)	94.20±6.80	83.50±6.20

Means±SE in the same row with different letters are significantly different (P<0.05). All the parameters were determined on day 30 postpartum.

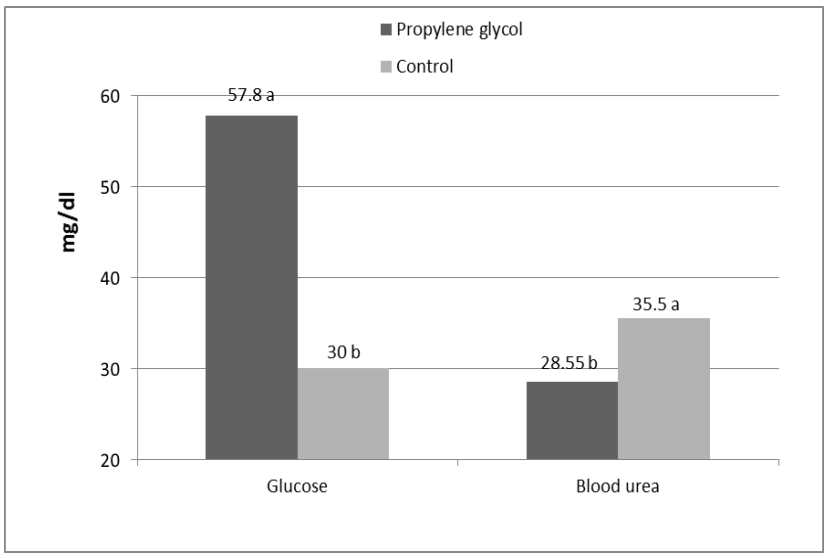

Figure 3. Impact of dietary propylene glycol (300 g/cow/day) on blood glucose and urea levels in Holstein cows

Table 7: Effect of dietary propylene glycol on reproductive performance of Holstein cows

Item	Control	Propylene glycol
First estrus (days)	57.0±9.0 [a]	35.30±2.99 [b]
Service per conception	2.60±0.51[a]	2.0±0.26 [b]
Days open	111.60±15.0 [a]	77.0±6.0 [b]

Means in the same row with different letters are significantly different (P<0.05).

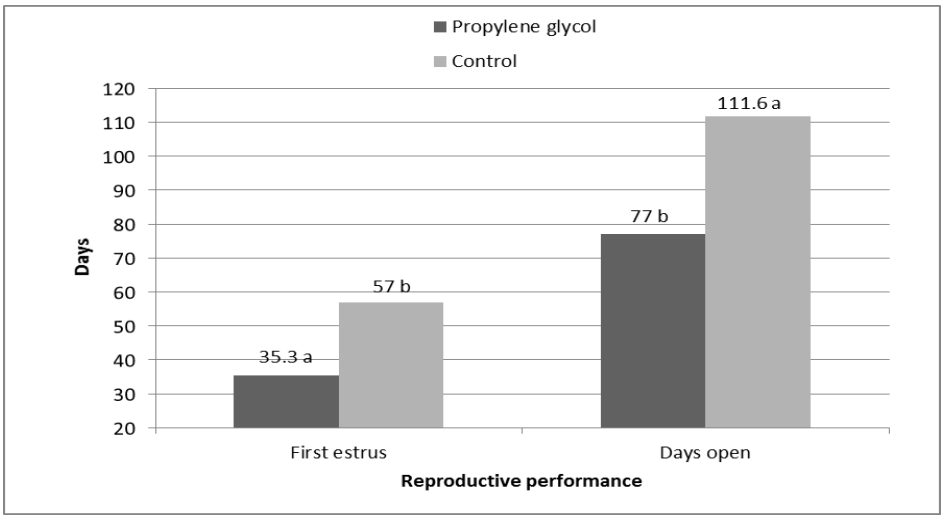

Figure 4. Impact of dietary propylene glycol (300 g/cow/day) on reproductive performance of Holstein cows.

4. DISCUSSION

The results of the present study showed that PG had a favorable effect on the BCS of Holstein cows. This indicated that PG decreased lipid mobilization postpartum. Similar results were reported by Grummer *et al*. (1994) and Knegsel et al., (2007) who described that PG tended to decrease body fat mobilization. PG rapidly supplies energy that decreased loss of BCS. PG acts to reduce adipose tissue lipolysis, which can be viewed as a metabolic adjustment by the cow to support parturition and lactation.

Our results showed that the supplementation of PG during the transition and lactation periods increased milk yield. This might be due to increased blood glucose levels and improving the energy status of the lactating cows. It is well known that glucose is metabolized in the mammary gland to lactose. The amount of milk yield depends on the quantity of lactose synthesized in mammary tissue. Because lactose has high osmotic pressure leading to drain of water and other milk constituents from circulation to mammary gland. This is compatible with Ballard et al., (2001) who have reported that feeding an energy supplement particularly PG improved milk yield.

Our results cleared that cows supplemented with PG had significantly higher milk protein % than those of the control group. Similar findings were reported by Hoedemaker et al., (2004) who found that PG increased milk protein% during 30-90 days of lactation. This might be due to the sparing effect of glucose for amino acids in the PG group. Also, this indicated that the energy status of cows supplemented with PG was better than those in the control group. Because during NEB some of the protein is metabolized to glucose instead of forming milk protein. Accordingly, this was explained by Sutton, (1989) who stated that milk protein% is positively associated with net energy balance. The low milk protein % in the control group may be due to subclinical ketosis (Duffield et al., 2009). There was a numerical increase in milk fat % in the control cows than those in the PG group. This was associated with lower BCS and higher fat mobilization of cows in the control group. Also, this indicated that the control cows might be exposed to subclinical ketosis more than the other group (Gillund et al., 2001). The association between milk fat % and hyperketonemia is due to the increased availability of BHBA and fatty acids for milk fat synthesis.

Our results showed a reducing effect of PG on serum NEFA prepartum. Also, PG decreased BHBA in blood and milk postpartum. These results are in agreement with McArt *et al.* (2011) who found that treatment of ketosis with PG reduced blood BHBA and increased milk yield. Additionally, Pickett et al., (2003) have indicated that pre-calving oral treatment with PG (300g/day) for 10 days, can reduce serum NEFA and ketone body concentrations. Also, our results showed a proportional relation between concentrations of BHBA in blood and milk. So, the determination of BHBA in milk could be reliable for the detection of ketosis in dairy cows.

Cows supplemented with PG had a lower blood urea level than the control cows and this was in agreement with Grummer *et al*. (1994). This indicated that the control cows suffered from severe NEB which leading to utilizing body protein for energy. As was expected, the blood glucose level was significantly higher in the PG group than the control one. This finding was similar to the results of Nielsen and Ingvartsen, (2004). This might be explained that PG is converted to pyruvate and eventually converted to glucose (Van Soest, 1994). In our study, the results

of blood glucose level, milk yield, and milk protein levels were associative. In which increase blood glucose levels resulted in increasing milk yield and milk protein. So, PG could be used as an antiketogenic energy supplement by increasing blood glucose concentrations (Kristensen and Raun, 2007) and lowering NEFA resulting in a decrease in serum BHBA concentrations (Grummer *et al.*, 1994 and Chung *et al.*, 2009).

There was no significant difference in liver enzymes (AST or ALT) between the two groups. The AST level was within the normal level in both groups. Gonzales *et al*. (2011) documented that AST activity higher than 100 IU/L is indicative of hepatic lesions. This indicated that the hepatocytes were not injured too much to release their enzymes into the serum.

The effect of PG on reproductive performance was valuable. PG decreased service per conception and shortened days open. This might be due to increased blood glucose level and decreasing intensity of NEB. It is well known that increasing blood glucose level elevates insulin hormone and insulin-like growth factors which have a positive effect on reproductive performance. These results are in agreement with McArt *et al*. (2012) who found that oral PG increased conception rate. They reported that cows treated with oral PG were 1.3 times more likely to conceive at first insemination than the control cows. It has also been postulated that the decrease in fat mobilization after PG administration may have beneficial effects on reproduction (Nielsen and Ingvartsen, 2004). The combined metabolic signaling of low blood glucose, insulin, and IGF-I concentrations. Also, the high BHBA delay the threshold of LH and FSH necessary for stimulation of ovarian follicles, estradiol production, and ovulation (Butler *et al.*, 2006). Low blood insulin concentrations are responsible for low IGF-I production from the liver. Together reduce the responsiveness of ovarian follicles to gonadotropins. Physiologically the metabolic and gonadotropin signals are interrelated. FSH stimulates granulosa cells in follicles to develop receptors for insulin, growth hormone and IGF-I. Insulin and IGF-I then provide the hormonal stimulus for the full development of preovulatory ovarian follicles (Rizos, et al., 2008).

5. CONCLUSIONS

Supplementation of Holstein cows with 300 g PG per day during the transition and early lactation periods increased milk yield and milk protein % through increasing blood glucose level and decreasing ketones. Also, PG improved the reproductive performance of early lactating cows. Finally, PG could be used to prevent ketosis and minimize loss of BCS after parturition.

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