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Performance, Metabolic Responses of Fresh Cows to Daily or Every Other Day Oral Drenching a Glucogenic Precursor

Research Article

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ABSTRACT

The delivery method of [continuous (CONT) *vs.* every other day or intermittent (INT)] a glucogenic precursor (GP) which was contained glycerin (500 g/kg), mono-propylene glycol (250 g/kg), calcium propionate (150 g/kg), niacin (1 g/kg) and sulfate-Co (350 mg/kg) on performance, selected blood metabolites and liver enzymes were evaluated. Twenty-four multiparous Holstein fresh cows were allocated in a completely randomized design (8 animals/each treatment) fed individually for a period of 14 days after calving. The experimental groups were: 1) no GP supplementation (CTR), 2) GP offered as an oral drench once a day (GP-CONT) on days 3, 4, 5, 6, and 7 after calving and 3) GP offered as an oral drench once a day intermittently (GP-INT) on days 3, 5, 7, 9, and 11 after calving. The amount of oral drenched GP was 1 kg/d. Blood samples were obtained on 5 and 14 days in milk. Dry matter intake (DMI) was increased (P=0.05) in GP-INT cows compared to GP-CONT but not control cows. The yield of fat corrected milk was greater in CTR cows than in GP supplemented cows (P=0.01). The milk protein and fat content were greater for GP-CONT and CTR groups, respectively. Milk somatic cell count was decreased in GP supplemented cows than in CTR cows (64.5 *vs.* 365×10^3/mL; P=0.02). Plasma total protein concentration was greater in GP-CONT group. Insulin concentration was increased for GP-CON animals on day 5 (P<0.05). Non-esterified fatty acids and β-hydroxy butyrate as well as liver enzymes were unaltered by treatments. However insulin sensitivity index was lower in supplemented treatments with GP rather than control group (P<0.05). In conclusion, the intermittent delivery of glucogenic precursor may be recommendable in fresh cows based on DMI criteria, which warrants further studies.

KEY WORDS fresh cow, glucogenic precursor, hepatic oxidation theory, liver enzymes.

INTRODUCTION

At the beginning of lactation, animals face a sudden and drastic increase in energy demand (Herdt, 2000). This demand is coupled with a decrease in feed intake, generally started in the dry period, and subsequently will cause to fat mobilization from the body stores in the form of nonesterified fatty acids (NEFA) to meet energy requirements (Gordon *et al*. 2013). Insulin resistance in combination with low plasma insulin concentration in the peripartum period will result in elevating plasma NEFA concentration and, in turn, severe fat inflow to liver could cause fatty liver and

hence ketosis will develop more severely contemporaneously (DeKoster and Opsomer, 2013).

The costs associated with ketosis include treatment of the disease, increased risk and treatment of other diseases, decreased milk production, poor reproductive performance, and a higher risk of culling in the first 30 d of lactation (McArt *et al*. 2012).

Different strategies such as management strategies (Amirabadi Farahani *et al*. 2017), intra-ruminal boluses technique (Compton *et al*. 2015) and glucogenic precursor's supplementation (DeFrain *et al*. 2004; Chagas *et al*. 2010) have been considered for improving fresh cows metabolic profiles. Different glucogenic precursors (GPs) such as propylene glycol (PG), glycerol or calcium propionate have been recommended as top-dressing or drenching in early lactating dairy cows to prevent ketosis (McArt *et al*. 2012; Bjerre-Harpøth *et al*. 2015). Recently Melendez *et al*. (2018) cleared that the combination of glucose precursors could improve energy status of cows in fresh period. Using different glucogenic precursors (e.g. propylene glycol, glycerol or calcium propionate) caused in an increase in the glucogenic status that was concurrent with a decrease in the plasma concentrations of β-hydroxy butyrate (BHBA) and NEFA (Formigoni *et al*. 1996; DeFrain *et al*. 2004).

The main effect of propionate in increasing glucose concentration is via changes hepatic PCK1 mRNA activity (Zhang *et al*. 2015). In contrast some restrictions may have been considered for GP supplementation, because of their potential toxic side effects including ataxia, salivation, hyperventilation and depression and also pulmonary vasoconstriction (Bertram *et al*. 2009) that this may be due to the dosage level of supplementation strategy. Therefore in addition to supplemented dosage level, different delivery methods of GP also could be an issue regarding the appetite control. As it has been cleared these products mostly increased blood glucose concentration via increased propionate concentration in rumen fluid (Czerkawski and Breckenridge, 1973).

The elevated propionate has potential to reduce intake based on hepatic oxidation theory (HOT); (Allen *et al*. 2009) through its hypophagic effect. Therefore expectedly greater propionate concentration reduce intake in continuous supplementing the GP sources. Moreover, every other day drenching of GPs may mitigate the labour problems and extra stress on fresh cows. Based on the literature mentioned above, we hypothesized that intermittent way of drenching the GP source may reduce hypophagic effect of propionate, as well as reducing the toxic compounds created in daily drenching of GP that consequently may improve intake, blood metabolites and liver function in fresh cows.

MATERIALS AND METHODS

Animal, design and treatment diets

The study was conducted at Mayan-Nojen dairy farm, Tehran, Iran. Animals were cared for according to the guidelines of the Iranian Council of Animal Care (1995). Multiparous (averaging ~ 3.5 parity) lactating Holstein cows (n=24) with 3 days in milk were assigned to treatments in a completely randomized design (8 cows per treatment). The animals were fed a basal diet formulated to meet nutrient requirements based on NRC (2001) recommendations. The basal diet and chemical compositions are presented in Table 1. The treatments were: 1) no GP supplementation (CTR), 2) GP offered as an oral drench once a day for 5 consecutive days (GP-CONT), and 3) GP offered as an oral drench once a day, 5 times in an every other day schedule (GP-INT). The GP was drenched at 3th, 4th, 5th, 6th, and 7th DIM for GP-CONT and at 3th, 5th, 7th, 9th, and 11th DIM for GP-INT. The GP consisted of glycerin (500 g/kg), monopropylene glycol (250 g/kg), calcium propionate (150 g/kg), niacin (1 g/kg) and sulfate-Co (350 mg/kg). The trademark of product was Z-Pro and it has been provided by Pajoohesh-Parvar Zayand Company (Isfahan, Iran). The oral drenching was performed one hour after morning feeding. Water was drenched in CTR animals throughout the study. The cows were fed individually with a total mixed ration (TMR) at 07:00 and 15:00 and had free access to water and NaCl white salt block. Orts were collected and weighed once daily at 0700 and the individual feeding rate was adjusted daily to achieve about 5% orts of intake. After finishing the experiment (14 days) cows were moved to the high-group pen and their milk production and milk components were recorded monthly. There were milked three times per day at 03:00, 11:00 and 19:00.

Experimental procedures and chemical analyses

The dry matter (DM) was determined for composites of feed and ort by drying at 60 ˚C for 48 h (AOAC, 2002). Dry matter intake was computed based on the 60 ˚C DM determinations for TMR and orts. Feed samples were taken weekly and composited to one sample. After drying, ingredients and TMR were ground through a 1 mm screen. Milk was sampled on days 3, 5, 8, and 11 after the first day of drenching and samples (from three consecutive milking) were analyzed for fat, protein, and somatic cell counts (SCC) (Milkoscan; Foss Electric, Hillerod, Denmark).

Blood was taken 4 h after morning feeding (approximately three hours after GP dosage) from the coccygeal vein of each cow at 5th and 14th DIM. Blood samples were heparinized and stored at 2 ˚C for about 6 h; samples were centrifuged (3000×g 4 ˚C, 20 min) and the harvested plasma was stored at –20 ˚C.

Table 1 Ingredients and chemical composition of basal diet

Ingredients	% of DM
Alfalfa hay	21.70
Corn silage	14.80
Beet pulp	9.10
Corn, ground	29.50
Soybean meal, 44% CP	6.30
Canola meal	7.35
Cottonseed meal	6.70
Rumen protected fat[1]	1.40
Vitamin-mineral mix[2]	0.70
Calcium carbonate	0.85
Sodium bicarbonate	1.15
Salt	0.40
Chemical composition	
Dry matter %	54.70
Crude protein, % of DM	16.00
Rumen degradable protein, % of CP	10.30
Rumen undegradable protein, % of CP	5.70
NEL[3], Mcal/kg	1.60
Neutral detergent fiber, % of DM	29.90
Acid detergent fiber, % of DM	19.10
Non-fiber carbohydrate, % of DM	43.60
Ether extract, % of DM	4.55
Ca, % of DM	0.59
P, % of DM	0.33

[1] RumiFat R100; it contains minimum 99.5% crude fat. Fatty acid composition: 71-73% C16:0, 4-6% C18:0, 16-18% C18:1, 3-5% C18:2. (Malaysia Palm, Co.).
[2] Provided per kg of DM: vitamin A: 500000 IU; vitamin D: 100000 of IU; vitamin E: 1000 mg; P: 9000 mg; Ca: 195000 mg; Mn: 2000 mg; Na: 55000 mg; Zn: 2000 mg; Fe: 2000 mg; Cu: 280 mg; Co: 100 mg; Br: 100 mg; Se: 1 mg and Antioxidant: 3000 mg.
[3] NEL: net energy for lactation; estimated using the NRC (2001) model.

The plasma then was analysed to determine the concentration of glucose (kit No. 93008), total protein (TP) (kit No. 9304), Blood urea nitrogen (BUN) (kit No. 93013), triglyceride (TG), and cholesterol using Pars Azmoon kits and associated procedures (Pars Azmoon Co., Tehran, Iran) as described previously (Kazemi-Bonchenari *et al.* 2017). The mentioned commercial laboratory reagents were used for measuring enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) using ELIS Aanalyzer (Auto Analyzer Hitachi 717, Japan). Other reagents were used to measure NEFA (Cat. No. FA 115, Randox Laboratories Ltd, UK), BHBA (Abbott Diabetes Care Ltd. Rang Road. Witney, Oxin, OX29 OYL., UK) and insulin (Cat. No. 2425-300A, MonobindInc, Lake Forest, CA, USA) using ELISA analyzer as mentioned above. Insulin sensitivity index was estimate by calculating RQUICKI (revise quantitative insulin sensitivity check index) using data from glucose, NEFA and insulin concentrations based on Holtenius and Holtenius, (2007) with the following equation (1/[log glucose + log insulin + log NEFA]).

Statistical analysis

Data were analyzed using Proc. Mixed in SAS (SAS, 2001).

The following model was used for variables which were repeated measurements over time:

$$Y_{ijk} = \mu + T_i + Z_k + ZT_{ik} + \varepsilon_{ijk}$$

Where:
Y_{ijk}: dependent variable.
μ: overall mean.
T_i: effect of treatment i.
Z_k: effect of time k (sampling dates).
ZT_{ik}: interaction between time k and treatment i.
ε_{ijk}: residual error.

Differences between least squares means were considered significant at $P < 0.05$, and differences were considered to indicate a trend toward significance at $0.05 < P < 0.10$.

Contrasts were used to evaluate control *vs*. GP and continuous vs. intermittent GP drenching.

RESULTS AND DISCUSSION

Animal performance

Although there was no significant effect of supplementing GP compared to un-supplemented treatment on DMI (P=0.12), but intake was increased with intermittent administration of GP compared to continuously GP feeding (P<0.05; Table 2).

Fat-corrected, but not raw, milk was greater (P<0.02; Table 2) in CTR cows compared GP supplemented cows. The milk protein and fat content were greater for GP-CONT and CTR groups (P<0.05) (Table 2). Accordingly, CTR yielded more milk fat (P<0.03).

Milk somatic cell count was decreased in GP supplemented cows than in CTR cows (P<0.02). The SCC was significantly decreased in the intermittent delivery of GP (P<0.05). There was no carry-over effect of treatments on productive variables such as milk production and components during month 1-3 after calving (Table 2).

Blood metabolites and insulin concentrations

The data for blood metabolites in the current study are presented in Table 3. The concentrations of NEFA, BHBA, TG, BUN, and albumin were not affected by treatments (P>0.05). Direct contrasts showed that blood glucose concentration was the greatest in GP-CONT delivery method (P<0.05) only on d 5 and thoroughout the experiment. Blood TP concentration was increased for GP-CONT delivery and cholesterol concentration was increased for GP-INT delivery method, respectively (P<0.05). The clearest response on insulin was revealed on d 5 and its concentration was increased by drenching GP in both kinds of deliveries compared to control (P<0.004; Table 4), and through the experiment GP-CON was more effective than GP-INT in increasing insulin. Among the measured enzymes GGT, ALT, ALP and AST did not show any significant difference among treatments (P>0.05) (Table 4). The insulin sensitivity index was reduced by GP supplementation compared with control group (P<0.05) (Table 5).

This study provides compelling evidence on effective use of glucogenic precursor within optimum choices of time schedule in early lactation. The rate of increase in feed intake postpartum lags behind the demands of lactation, leading to a period of negative energy balance (Gordon *et al.* 2013). Early lactating dairy cows must accommodate a tremendous increase in energy demand by the mammary gland for milk production with increasing DMI (Nielsen and Ingvartsen, 2004).

Therefore, cows should be encouraged to maximize their intake during early lactation. In the present study, DM was increased with an intermittent drenching of GP but not with the continuous delivery method.

To perceive and possibly prevent hypophagia, we need to be able to quantify the linkages between mechanisms of intake regulation, metabolism, and the immune system in the dairy cow (Ingvartsen, and Andersen, 2000). The basal diet was similar in the present study and the only variable factor was offering GP method which was continues versus intermittent. Although the volatile fatty acids (VFAs) have not been measured in the present studies, as it has been previously cleared, the main glucogenic precursor product in the rumen is probably propionate when glycerol (Wang *et al.* 2009) or when PG has been offered (Czerkawski and Breckenridge, 1973). Based on the HOT, among the fuels metabolized by the ruminant liver, propionate is likely a primary satiety signal because its flux to the liver increases greatly during meals (Allen *et al.* 2009). From the other hand, propionate may changing the hepatic PCK1 mRNA activity that subsequently could positively affect glucose concentration (Zhang *et al.* 2015). This contradictory effect of propionate may be a function of its concentration in rumen fluid of fresh dairy cows. Because the greatest glucose concentration was observed in GP-CONT delivery method, probably the greatest propionate has been expected to be produced in this treatment. Moreover, the greater observed insulin in GP-CONT treatment may be a factor in lower DMI. Leury *et al.* (2003) showed that hyper insulinemic-euglycemic clamps increased plasma insulin concentration and decreased DMI 33% in fresh cows. However, it is not likely the reason why DMI increased in GP-INT, because the insulin concentration was similar between CTR and GP-INT groups. Allen *et al.* (2005) illustrated when glucose demand is high (e.g., early lactation), providing the liver with propionate results in increased gluconeogenesis, in turn enhancing hepatocyte ATP consumption and increasing DMI. Accumulation of tricarboxylic acid cycle intermediates from a reduction of gluconeogenesis from propionate will likely increase oxidation of acetyl CoA, suppressing DMI despite the reduction in plasma NEFA. Accordingly, considering the similar concentrations of NEFA and BHBA across treatments, it appears that continuous delivery of GP has been caused the tricarboxylic acid cycle intermediates to increase, in turn, resulted in lower DMI compared to GP-INT group. Because the second blood sample had been taken at 14th DIM (3 d after last GP drench), we are not able to see how and why DMI was lower for CTR and GP-CONT group based on blood metabolites, which warrants more experiments. Different results for milk yield and composition were achieved when the GP was used in dairy cows. The milk was increased in some studies which the GP has been used in dairy cows (Pickett *et al.* 2003), but in some others had a tendency towards unchanged or decreased milk yields when PG was allocated (Dhiman *et al.* 1993; Shingfield *et al.* 2002).

Table 2 Effect of continuous or intermittent glucogenic precursor (GP) delivery on performance of dairy cows in first two weeks and first three months of lactation

Item	Treatments			SEM	P-value	Contrast	
	CTR	GP-CONT	GP-INT			CTR *vs.* GPs	GP-CONT *vs.* GP-INT
Week (1 to 2)							
Dry matter intake (DMI), kg/d	14.95	14.69	16.10	0.53	0.12	0.45	0.05
Milk yield, kg/d	39.4	35.45	37.59	2.05	0.41	0.26	0.46
Fat-corrected milk (FCM), kg/d	48.7	39.05	42.22	2.74	0.06	0.02	0.42
Fat, %	5.56	4.66	4.88	0.17	0.003	0.001	0.38
Protein, %	3.28	3.38	3.22	0.04	0.03	0.77	0.01
Fat, kg/d	2.19	1.65	1.81	0.13	0.02	0.01	0.42
Protein, kg/d	1.29	1.19	1.2	0.06	0.58	0.3	0.94
Somatic cell count, ($\times 10^3$/mL)	365.7	137.25	64.51	38.99	0.08	0.02	0.71
Month (1 to 3)							
Milk yield, kg/d	43.79	46.87	44.29	1.62	0.36	0.39	0.26
FCM, kg/d	39.89	43.27	40.67	1.42	0.22	0.26	0.19
Fat, %	3.43	3.49	3.45	0.07	0.86	0.68	0.72
Protein, %	2.83	2.89	2.81	0.05	0.53	0.79	0.28
Fat, kg/d	1.49	1.63	1.52	0.05	0.19	0.22	0.18
Protein, kg/d	1.23	1.34	1.24	0.04	0.17	0.31	0.12

CTR: no GP supplementation; GP-CONT: GP offered as an oral drench once a day on days 3, 4, 5, 6, and 7 after calving and GP-INT: GP offered as an oral drench once a day and every other days on days 3, 5, 7, 9, and 11 after calving.
SEM: standard error of the means.

Saleem *et al*. (2018) concluded that glycerol supplementation could improve dairy buffaloes through improving nutrients digestibility then consequently could affect milk yield and composition. Piantoni and Allen (2015), reported that glucose may be more limiting than protein for milk production during the period immediately after calving; glucose infusion, however, lasted 12 days in that study which is far from our study. In addition, glucose concentration on d 14 was not influenced by treatments which explains why a milk response was not observed in this experiment.

The greater fat-corrected milk (FCM) yields from cows fed the control diet were in agreement with DeFrain *et al.* (2004) and Fisher *et al*. (1973). Effects of treatments on yields of milk fat had been attributed to the decrease in the ruminal acetate to propionate ratio in DeFrain's experiment. Griinari and Bauman (2006) stated that a decrease in milk fat yield reduces the energy demand and thus has the potential to provide the cow in early lactation with more latitude to adapt and accommodate metabolic demands. Therefore, such strategies could be carried out as a tool to prevent ketosis and milk fat reduction, which warrants more research. Moreover, the mode of action through improvement of nutrients digestion caused by glucogenic precursor might be a reason for improvement in animal productivity after freshening (Saleem *et al*. 2018).

Considering the blood metabolites, the NEFA concentration was not affected by treatments. It has been shown NEFA and plasma insulin concentrations are inversely related to days (Allen, 2014), but we did not find the similar relationship in this study.

The BHBA as well as NEFA, are indicators of energy balance in early lactating dairy cows. It has been shown that the effect of glucogenic precursors on energy balance indices in blood is different, probably due to the physiological status of animal, dosage amount of source, and time of blood sampling after dosing (Nielsen and Ingvartsen, 2004). Cholesterol concentration in blood was increased in GP-INT treatment.

Formigoni *et al*. (1996) reported the higher total cholesterol levels in the plasma of PG treated cows after parturition. Increased gluconeogenesis precursors in the liver could promote cholesterol synthesis and/or release by the hepatocytes (Formigoni *et al*. 1996).

In addition to the positive effects of GP administration in both delivery methods, greater cholesterol level in intermittent compared to continuously offered cows was probably due to greater intake and then greater energy available for the animal. In addition to the mechanisms discussed above, Saleem *et al.* (2018) postulated that glycerol which is a glucose precursor in fresh buffaloes improved some nutrients digestibility and hence could improve animal productivity.

Insulin was increased by offering GP in the present study. Studer *et al*. (1993) reported that mean plasma insulin concentrations were significantly increased by PG offering in dairy cows. Volatile fatty acids appear to be more potent than glucose with respect to stimulating insulin secretion (Manns *et al*. 1967; Brockman, 1978). The concentration of VFA is greater in dairy cows offered different glucogenic precursors compared to untreated ones (Czerkawski and Breckenridge, 1973).

Table 3 Effect of continuous or intermittent glucogenic precursor (GP) delivery on blood metabolites of dairy cows at 5 and 14 dry matter intake (DIM)

Item	Treatments			SEM	P-value	Contrast	
	CTR	GP-CONT	GP-INT			CTR *vs*. GPs	GP-CONT *vs*. GP-INT
Glucose, mM							
5 DIM	2.40	3.46	3.13	0.19	0.07	0.008	0.007
14 DIM	2.69	3.17	3.01	0.33	0.43	0.23	0.21
Average	2.54	3.32	3.06	0.71	0.01	0.005	0.004
Beta-hydroxy butyrate, mM							
5 DIM	0.58	0.37	0.47	0.08	0.22	0.13	0.38
14 DIM	0.46	0.57	0.74	0.14	0.40	0.27	0.42
Average	0.52	0.47	0.61	0.09	0.59	0.85	0.32
Non-esterified fatty acids, mM							
5 DIM	1.14	1.08	1.22	0.15	0.80	0.97	0.51
14 DIM	1.13	0.97	1.30	0.24	0.63	0.99	0.34
Average	1.14	1.03	1.26	0.13	0.47	0.96	0.91
Total protein, mg/dL							
5 DIM	6.94	8.08	7.40	0.23	0.01	0.01	0.004
14 DIM	7.95	8.10	7.63	0.21	0.51	0.81	0.71
Average	7.48	8.04	7.51	0.18	0.04	0.18	0.03
Albumin, mg/dL							
5 DIM	4.0	4.28	4.05	0.17	0.48	0.44	0.26
14 DIM	4.27	4.18	4.29	0.24	0.91	0.90	0.83
Average	4.14	4.20	4.16	0.15	0.88	0.75	0.66
Blood urea nitrogen, mg/dL							
5 DIM	23.31	18.85	24.25	2.72	0.37	0.60	0.27
14 DIM	25.25	23.19	26.12	2.56	0.58	0.76	0.45
Average	24.28	21.56	25.18	1.82	0.22	0.57	0.19
Triglyceride, mg/dL							
5 DIM	45.16	51.12	63.0	10.7	0.46	0.23	0.25
14 DIM	42.50	34.12	32.17	6.25	0.78	0.51	0.49
Average	43.50	42.64	47.75	8.56	0.35	0.40	0.52
Insulin, µIU/mL							
5 DIM	8.83	18.37	12.28	1.65	0.002	0.004	0.16
14 DIM	8.11	10.56	10.12	1.69	0.44	0.28	0.39
Average	8.43	14.47	11.18	1.97	0.01	0.01	0.01
Cholesterol, mg/dL							
5 DIM	113.2	132.5	124.7	9.21	0.36	0.87	0.56
14 DIM	123.7	148.5	152.3	8.43	0.05	0.18	0.98
Average	118.8	137.6	139.2	6.24	0.02	0.33	0.64

CTR: no GP supplementation; GP-CONT: GP offered as an oral drench once a day on days 3, 4, 5, 6, and 7 after calving and GP-INT: GP offered as an oral drench once a day and every other days on days 3, 5, 7, 9, and 11 after calving.
SEM: standard error of the means.

Table 4 Effect of continuous or intermittent glucogenic precursor (GP) delivery on liver enzymes of dairy cows at 5 and 14 dry matter intake (DIM)

Item	Treatments			SEM	P-value	Contrast	
	CTR	GP-CONT	GP-INT			CTR *vs*. GPs	GP-CONT *vs*. GP-INT
Aspartate aminotransferase (AST), IU/L							
5 DIM	123.12	108.85	123.25	15.58	0.77	0.71	0.53
14 DIM	100.375	112.42	118.75	15.37	0.69	0.43	0.59
Average	111.75	110.53	121.10	10.81	0.77	0.76	0.94
Alkaline phosphatase (ALP), IU/L							
5 DIM	95.42	88.20	70.90	19.61	0.70	0.68	0.96
14 DIM	44.57	65.50	44.83	9.98	0.30	0.41	0.17
Average	70.10	76.41	57.54	12.90	0.44	0.92	0.58
Alanine aminotransferase (ALT), IU/L							
5 DIM	28.62	27.0	31.70	2.41	0.61	0.96	0.65
14 DIM	33.62	27.12	31.12	5.19	0.65	0.53	0.38
Average	31.12	27.10	31.32	2.35	0.52	0.57	0.32
Gamma-glutamyltransferase (GGT), IU/L							
5 DIM	17.75	19.0	12.25	3.99	0.50	0.68	0.84
14 DIM	22.0	29.0	14.66	3.64	0.43	0.98	0.49
Average	19.87	23.55	13.28	3.52	0.27	0.90	0.36

CTR: no GP supplementation; GP-CONT: GP offered as an oral drench once a day on days 3, 4, 5, 6, and 7 after calving and GP-INT: GP offered as an oral drench once a day and every other days on days 3, 5, 7, 9, and 11 after calving.
SEM: standard error of the means.

Table 5 Effect of continuous or intermittent glucogenic precursor (GP) delivery on insulin sensitivity index (RQUICKI)

Item	Treatments			SEM	P-value	Contrast	
	CTR	GP-CONT	GP-INT			CTR *vs*. GPs	GP-CONT *vs*. GP-INT
RQUICKI[1]							
5 DIM	0.72	0.54	0.59	0.02	0.04	0.01	0.23
14 DIM	0.71	0.66	0.62	0.03	0.05	0.02	0.54
Average	0.72	0.59	0.61	0.02	0.02	0.01	0.74

[1] RQUICKI; revised quantitative insulin sensitivity check index; was estimated based on (Holtenius and Holtenius, 2007).
CTR: no GP supplementation; GP-CONT: GP offered as an oral drench once a day on days 3, 4, 5, 6, and 7 after calving; GP-INT: GP offered as an oral drench once a day and every other days on days 3, 5, 7, 9, and 11 after calving and DIM: dry matter intake.
SEM: standard error of the means.

Therefore, the greater total VFA concentration in the rumen and especially greater propionate ratio (Manns *et al*. 1967) may have caused to greater insulin concentration. The results show that, regardless of the delivery method of GP, oral drenching of GP increased insulin concentration which between the methods, continuous delivery caused to the greater value. The insulin sensitivity index was reduced by GP supplementation in comparison with control group. However this parameter did not influence with the method of drenching. Based on Holtenius and Holtenius (2007) three different factors contributed to estimation the insulin sensitivity index (i.e. glucose, NEFA and insulin concentration).

Looking in to results obtained in the present study, although NEFA concentration was constant among treatments, different insulin as well as glucose concentrations in blood caused to achieve different insulin sensitivity index. The results of the present study clear that GP supplementation could modify insulin sensitivity in fresh dairy cows which granted to do more works. Among different enzymes measured in the present study, none of the enzymes did differ among treatments. This shows that liver did not respond to continuous or intermittent drenching of supplement.

CONCLUSION

Compared to intermittent administration of GP into fresh dairy cows, continuous administration increased blood glucose concentration, however reduced DMI. Milk protein positively was affected by continuous dosing as well. The intermittent administration of glucogenic precursor in postpartum dairy cows caused to greater daily feed intake compared to daily administering. In conclusion, intermittent administration of glucogenic precursor may be recommendable in fresh cows to prevent severe negative energy balance by improving intake and temporary reducing FCM production. Further researches are granted to evaluate these two delivery methods for different glucogenic precursors in this critical period of time in dairy cows.

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