

## Impacts of Different Precursors of Glucogenic and Mineral Supplements on Metabolic Status of Holstein Fresh Cows

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**Abstract:** The objectives of this study were to determine whether liquid feeding or TMR feeding of glucogenic and mineral supplements can improve metabolic status and plasma metabolites in Holstein fresh cows. Multiparous cows (n = 24), second lactating cows (n = 24) and first lactating cows (n = 24) were used in a completely randomized block design, immediately after parturition with 3 weeks experimental period treatments were: Control Treatment (CONT) without any additive on water or TMR (CONT), Drenching treatment (DRCH) include: 40 L water contained: 2 kg of calcium propionate, propylene glycol, potassium chloride, magnesium sulfate, sodium phosphate, sodium chloride, sodium bicarbonate, choline chloride and methionine, feeding treatment (FEED) contained 2 kg of drenching diet contents that were mixed with TMR, compound diet (DRFD), 20 L water contained 1 kg of drenching treatment plus 1 kg of these materials that mixed to TMR. Treatments had significant effects on Ca, Mg and P concentrations of plasma (p<0.05) and these metabolites had more concentrations in DRCH treatment than others. DRCH treatment had positive effects on negative energy balance and plasma NEFA and  $\beta$ -HBA concentrations. Plasma glucose had increased significantly by treatment. Therefore, addition of these glucogenic precursors and minerals on water of fresh cows resulted in optimize animal metabolic status in postpartum period.

**Key words:** Fresh cows, glucogenic precursors, mineral supplements, negative energy balance, blood metabolites, Iran

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### INTRODUCTION

It is widely recognized that intake in the immediate postpartum period lags behind that needed to support milk production such that the cow experiences negative energy and protein balance for several weeks following the initiation of lactation. To cope with the large increase in nutrient demand associated with milk production during this time, the cow experiences a multitude of metabolic adaptations (Bell, 1995; Ghorbani *et al.*, 2007).

At the initiation of lactation, the demand for glucose for lactose production increases markedly and is partially met by an increase in gluconeogenesis as well as a decrease in glucose oxidation (Bell, 1995). The contribution of AA to gluconeogenesis has been considered important during early lactation in the dairy cow (Bell, 1995) but supportive evidence has come from observations either *ex vivo* or *in vitro* (Drackley *et al.*, 2001). Because of inability of cow in transition period to have increased amount of DMI, managers often used

drenching or pastes to provide glucose precursors to prevent hypocalcemia, ketosis and other metabolic disorders (Van Knegsel *et al.*, 2007; Studer *et al.*, 1993; Goff and Horst, 1993) and synchronously could maintain high productive and reproductive efficiencies (Miyoshi *et al.*, 2001). Recent researches (Stokes and Goff, 2001) indicated that feeding a large amounts of water contained electrolytes to fresh cows in order to prevent metabolic disorders but researches on this field are very limited (Enemark *et al.*, 2009).

Drinkings a large amount of water by cows, immediately after parturition have two goals: cows in calving time had lost about 60 L of conceptus fluids that cause electrolyte imbalances, thus drenching large amounts of water contained minerals would be replaced electrolytes losts, rumen fill may results to rapid movement of rumen to down parts of abdominal cavity, so can prevent abomasal displacement (Enemark *et al.*, 2009). Propyleneglycol have enriched of energy (4.7 Mcal NEL L<sup>-1</sup>) that could provide energy requirement

of dairy cows in transition period. Propylene glycol easily metabolized and absorbed in the rumen. Total 50% of propylene glycol, metabolized after 1-2 h post feeding and almost 80-90% of remained, metabolized after 3 hand transfer to the liver and converted to glucose via gluconeogenesis pathway (Miyoshi *et al.*, 2001). Some researchers (Christensen *et al.*, 1997; Pickett *et al.*, 2003; Miyoshi *et al.*, 2001) reported that propyleneglycol could stimulate insulin secretion and decreased NEFA and keton bodies in plasma therefore can increase milk production.

Researchers determines that calcium propionate, provide a rapidly absorb of Ca and prevents clinical and subclinical hypocalcemia (Goff *et al.*, 1996; Pehrson *et al.*, 1998; Melendez *et al.*, 2002) and can minimize adipose tissue mobilization as a glucogenic precursor thus fat deposition in liver and concentrations of NEFA and  $\beta$ -HBA in plasma can decreased (Fonseca *et al.*, 1998; Cozzi *et al.*, 1996; Christensen *et al.*, 1997) or diminish losses of BCS (Lucci *et al.*, 1998; Formigoni *et al.*, 1996).

Magnesium sulfate, provides Mg to maintain natural homeostasis of Ca at parturition due to its role on secretion of PTH and inductive effects of PTH on Ca absorption in small intestine (Enemrak *et al.*, 2009). Potassium Chloride can provide K that can maintain intracellular cation also have important role in acid-base balance and accretion of water in the body and have powerfull effect on  $Mg^{2+}$  absorption. K reserves are limited in the body and needs to provide, daily (Ammerman and Goodrich, 1983).

Choline have three methyle group and four amine group that participate in fat transition. Choline provides labile methyle groups for transmethylation reactions and is essential for synthesis of phosphatydile choline of plasma memberane of cells (Hartwell *et al.*, 2000; Zahra *et al.*, 2006). Choline can increase milk fat syathesis (Erdman *et al.*, 1984) and used for care of fatty liver at postpartum (Cooke *et al.*, 2007). It seems that choline chloride acts as a methyle donor for methyle sparing in milk fat syathesis (Drackley *et al.*, 2001).

The objectives of this study were to compare different methods of adding glucogenic precursors and mineral supplements in early postparturient period and goals of this study were ameliorate severity of NEB after parturition, improving metabolic profile of plasma during 21 days in milk.

## MATERIALS AND METHODS

**Cows and experimental design:** About 72 cows immediately after parturition were housed in individual

boxes for 24 h and randomly assigned based on their parity number (first lactating, n = 24; second lactating, n = 24; third lactating, n = 24), to four experimental treatments. After 24 h, cows were hosed at freestalls and received one TMR for 3 time a day (0730, 1300 and 1800 h).

**Experimental treatments.** Treatments consists of: Control diet without any supplements (CONT), Drenching treatment (DRCH) contains: 2 kg of glucogenic and mineral supplement: 500 g, calcium propionate; 500 g, sodium phosphate; 150 g, sodium chloride; 150 g, sodium bicarbonate; 25 g, choline chloride and 12 g, methionine that cows received these materials with 40 L water on two time. Water temperature was 40°C.

In first time, 1 kg of these materials dissolved in 20 L water and offered to cows and at second time, another 20 L, force fed to cows with esophageal tube. Feeding by mixed with TMR (FEED): 2 kg of those materials were mixed with TMR and cows fed them three time a day, compound treatment (DRFD): 1 kg of those materials dissolved on 20 L water and 1 kg mixed with their TMR.

**Sample collection.** Feed intake and orts were recorded daily throughout the experiment. Weekly samples of TMR and orts were collected and stored at -20°C for later analysis.

Blood samples were collected from each cows on days 2, 4, 7, 14 and 21 after parturition about 4-5 h after morning meal via tail venipuncture and immediately centrifuged at 1800×g for 15 min to obtain plasma which were then stored at 20°C for later analysis but blood glucose were measured immediately after blood collection via portable glucose assessment (Gloc-Trand 2) and by Accu-Check kit (Germany). Plasma NEFA and BHBA were measured enzymatically via Randox kit. Ca, P and Mg were measured using Pars Azmoon kits with spectrophotometry (Perkin-Elmer-35) (Table 1 and 2).

**Statistical analysis:** All data were analyzed using a MIXED procedure of SAS Institute (2004). Days of blood collection were as repeated measurements. Treatment was considered as a fixed effect and days of sampling (relative to calving) as the repeated measure.

Data were analyzed as a completley randomized block design. Comparison of means between treatments were done by Tukey test and p<0.05 were declared as significant.

**Table 1: Experimental ingredients of diets (percentage of DM basis)<sup>1</sup>**

Ingredients	Experimental diets	
	Close-up	Fresh cow
Alfalfa hay	32.96	21.74
Corn silage	23.60	16.94
Wheat straw	-	0.72
Beet pulp	4.82	3.99
Barley grain, ground	11.12	16.24
Com grain, ground	5.76	9.92
Whole cottonseed	3.64	6.22
Cottonseed meal	2.48	3.24
Canola meal	3.65	2.39
Soybean meal	5.07	7.81
Fish meal	1.52	2.05
Corn gluten meal	0.86	1.61
Whole soybean	-	2.27
Vegetable oil	1.05	1.78
Salt	-	0.36
Sodium bicarbonate	-	0.88
MgO	-	0.42
CaCO <sub>3</sub>	1.4	0.36
Mono calcium phosphate	-	0.31
Vitamin supplement	0.45	0.62
Ammonium sulfate	0.44	-
Calcium chloride	0.76	-
Mg sulfate	0.43	-

<sup>1</sup>Contains: 1800000 IU, Vit. A; 400000 IU, Vit. D<sub>3</sub>; 8000 IU, Vit. E; 3000 Mg, Antioxidant

**Table 2: Chemical composition of experimental diets (Percentage of DM basis)**

Items	Diets	
	Close-up	Fresh cow
NEI (Mcal Lg <sup>-1</sup> )	1.54	1.69
CP (%)	15.80	17.50
RDP (% of CP)	11.50	11.60
RUP (% of CP)	4.30	5.90
NDF (%)	37.00	32.00
hNDF (%)	27.40	19.20
ADF (%)	25.00	21.00
NFC (%)	36.40	39.00
EE (%)	3.80	5.30
Ca (%)	1.50	1.00
P (%)	0.40	0.50
DCAD (meq kg <sup>-1</sup> )	-55.00	247.00

**RESULTS AND DISCUSSION**

**Calcium:** Least square means of plasma Ca between treatments were 8.98, 8.06 and 8.30 mg dL<sup>-1</sup>, respectively (Table 3). Effects of treatments were significant (p<0.05). DPHC treatment had highest concentration of Ca compared with other treatments. Ca supplements used in this study resulted to increasing plasma Ca and this increase improve Ca status of cows during whole period of the experiment (day 21st). This incremental trend in drenching treatment may resulted from increased Ca<sup>++</sup> and finally absorption of these ioness increased. Moreover, availability of these Ca salts due to their osmotic nature resulted to stimulation of esophageal groove and passage

of Ca to the abomasum therefore, it's prevent dilution of Ca solutions in the rumen and resulted to increasing gradients of Ca concentrations in abomasum and finally, passive transport of Ca in small intestine increased (Goff and Horst, 1993).

However, in DRFD treatment, increased concentration of Ca could be related to increased concentration of luminal Ca and resulted in optimum passive transport of Ca to extracellular fluid (Goff and Horst, 1993). Capacity of passive transport of Ca is unlimited and it is unrelated to stimulation of 1, 25- (OH)<sub>2</sub> D<sub>3</sub> thus Ca<sup>++</sup> absorption was increased linearly via increasing luminal Ca (Breves *et al.*, 1995).

In agreement with the findings, Dhiman and Sasidharan (1999) suggested that using Ca supplement after parturition increased Ca concentration of plasma in comparison of control group. Also, Oetzel (1996) reported that Ca concentration of plasma was increased about 0.72 mg dL<sup>-1</sup>, 1 day after feeding four doses of calcium chloride gel. Peralta *et al.* (2011), drenched calcium propionate and propylene glycol via a pumping system and reported significant increase in plasma Ca, 12 h after calving. Goff *et al.* (1996) reported higher level of plasma Ca, 12 h after two doses of Ca propionate at parturition. In contrast to the findings Melendez *et al.* (2002) reported that supplemental Ca, 12 h after calving, did not have significant effects on plasma Ca during 12 day in milk.

**Phosphorus:** Least square means of plasma P between treatments were 5.26, 6.10, 4.76 and 4.97 mg dL<sup>-1</sup>. Treatments were significantly different (p<0.05). Larsen *et al.* (2001) determined that there was a positive correlation between Ca and P in healthy and milk fever cows. Normal range of plasma P in dairy cattle were 4-8 mg dL<sup>-1</sup> (NRC, 2001). Mineral salts used in this trial such as sodium phosphate, resulted to significant increase in plasma P but this increase was highest in DRCH treatment and cows maintained these concentration during 3 weeks of lactation. Because normal range of plasma Ca were detected in this study, a similar result could be suggested for plasma P. Since, when Ca was in normal level, secretion of PTH, decreased and therefore, P excretion via saliva and urine, decreased. Also, absorption of P via diets and reabsorption of P via saliva increased (Goff, 1999) that are in agreement with the findings. In contrast with the findings, Dhiman and Sasidharan (1999) did not report any increase in plasma P after feeding of Calcium chloride gel. Melendez *et al.* (2002) reported that feeding and injection of Ca and energy supplements in fresh cows did not have significant effects on plasma Peralta *et al.* (2011) used a package contained caproponate, propylene glycol and

Table 3: Least square means of plasma metabolites<sup>1</sup>

Items	CONT <sup>1</sup>	DRCH	FEED	DRFD	SEM <sup>2</sup>	p-value <sup>3</sup>			
						T	B	D	B×T
Ca (mg dL <sup>-1</sup> )	8.28 <sup>b</sup>	8.98 <sup>a</sup>	8.06 <sup>c</sup>	8.30 <sup>b</sup>	0.081	0.0001	0.0015	0.0001	0.0001
P (mg dL <sup>-1</sup> )	5.26 <sup>b</sup>	6.10 <sup>a</sup>	4.76 <sup>c</sup>	4.97 <sup>c</sup>	0.096	0.0001	0.0002	0.0001	0.0001
Mg (mg dL <sup>-1</sup> )	2.12 <sup>b</sup>	2.56 <sup>a</sup>	1.99 <sup>b</sup>	2.05 <sup>b</sup>	0.052	0.0001	0.8570	0.0001	0.0001
Glucose (mg dL <sup>-1</sup> )	58.77 <sup>b</sup>	64.48 <sup>a</sup>	56.35 <sup>b</sup>	55.71 <sup>b</sup>	1.530	0.0001	0.0001	0.0001	0.1660
NEFA (mmol L <sup>-1</sup> )	0.62 <sup>c</sup>	0.53 <sup>b</sup>	0.79 <sup>a</sup>	0.68 <sup>bc</sup>	0.035	0.0001	0.0001	0.0001	0.0001
βHBA (mmol L <sup>-1</sup> )	0.59 <sup>a</sup>	0.51 <sup>b</sup>	0.51 <sup>c</sup>	0.53 <sup>b</sup>	0.021	0.0001	0.0001	0.0706	0.0001

<sup>1</sup>CONT = Control treatment, DRCH = Drenching treatment, FEED = Feeding precursors with TMR, DRFD = Drenching plus feeding treatment; <sup>2</sup>Standard error mean; <sup>3</sup>T = Treatment, B = Block, D = Day

other mineral, using a pumping system and did not shown any significant increase in plasma P. It was seemed that cause of these contrasts is related to using 200 g sodium phosphate and cows enable to maintain plasma P via absorption dietary P or via reabsorption of P from kidneys (Goff *et al.*, 1986; Oetzal, 1988). In agreement with the findings, Gregory *et al.* (1993) reported that by using calcium chloride, plasma P was increased.

**Magnesium:** Least square means of Mg between treatments were: 2.16, 2.56, 1.99 and 2.05 mg dL<sup>-1</sup>. Treatments were significantly different (p<0.05). Natural range of plasma Mg is between 1.8-2.4 mg dL<sup>-1</sup> (Goff, 1999). Immediately after parturition, active transport for Mg from rumen papillae would be influenced by dietary potassium and unknown factors that prevent effective absorption of Mg by this way. A second way for Mg absorption, act only in high concentration of Mg in ruminal fluid. In high concentration of Mg in ruminal fluid, gradient of Mg conducted to extracellular fluids (Martens and Schweigel, 2000). This passive transport, influenced by Mg solubility and did not affected by potassium toxicity therefor, passive transport at claving, that cows faces with low DMI and so supply of dietary Mg is low could resulted to use of Mg gels or drenching of 200-400 mL of Mg sulfate solution (Goff, 1999, 2006).

In contrast with our findings compared with others (Dhiman and Sasidharan, 1999; Melendez *et al.*, 2002) researchers supplied 250 g Mg sulfate that showing increased absorption of Mg via rumen papillae, specially in DRCH treatment.

**Glucose:** Least square means of plasma glucose between treatment were 58.77, 64.48, 56.35 and 55.71 mg dL<sup>-1</sup>, respectively (Table 3). There were a significant difference between treatments (p<0.05). Plasma glucose concentration have been decreased 25% at 1st week of lactation and gradually increased at the 2nd week of lactation. This increase would be related to improving in DMI and energy status of cows (Bertics *et al.*, 1992; Skaar *et al.*, 1989; Studer *et al.*, 1992). In agreement with

the findings, Lien *et al.* (2010) suggested propylene glycol that drenched one time a day resulted to increase plasma glucose. Grummer *et al.* (1994), Miyoshi *et al.* (2001) and Juchem *et al.* (2004) found that feeding propylene glycol in transition period caused to significantly increased in plasma glucose. Propylene glycol converted to propionic acid in the rumen (Grummer *et al.*, 1994) and converted to glucose in the gluconeogenesis pathway. In agreement with the findings in FEED treatment, Chung *et al.* (2009) and Castaneda-Gutierrez *et al.* (2009) reported any effects of feeding propylene glycol with TMR on plasma glucose. McNamara and Valdez (2005) did not shown any effects of feeding Ca propionate on plasma glucose.

**NEFA and β-HBA:** Least square means of NEFA and β-HBA between treatments were: 0.62, 0.53, 0.79, 0.68 mmol L<sup>-1</sup> and 0.59, 0.51, 0.51 and 0.53 mmol L<sup>-1</sup>, respectively. McFadden *et al.* (2010) reported that drenching propylene glycol or a package contained glucogenic precursors plus minerals resulted to decreased NEFA and β-HBA. Enemark *et al.* (2009) suggested drenching Ca propionate, potassium chloride and magnesium sulfate in 20 L water had significantly effects on NEB and decreased concentration of β-HBA and NEFA had trend toward decreased.

Since, chloride is a lipotropic factor, it seems to have beneficial effects on adipose tissue and liver metabolism (Piepenbrink and Overton, 2000). There was determined a significant interaction between treatment and time for plasma concentration of NEFA and ratios of NEFA to cholesterol indicated that increase in NEFA to supplemented with protected choline were lower than control group (Pinotti *et al.*, 2003).

Choline as a methyle acts in synthesis of carnitine that is essential for fatty acid oxidation therefore, decreased level of β-HBA in cows received choline, compared with CONT treatment was due to supplying role of choline in fatty acid metabolism (Pinotti *et al.*, 2003). The findings were in agreement with Piepenbrink and Overton (2000) that were shown protected choline, in periparturient period could decreased deposition of FA in the liver and increased amounts of glycogen.

## CONCLUSION

Result of this study showed that drenching of glucogenic precursors and mineral supplements, immediately after parturition had better results than other methods such as feeding with drenching and resulted to significant increase in plasma concentrations of Ca, P and Mg. These results would be related to decreased incidence of hypocalcemia, milk fever, hypomagnesemia and displaced abomasum and thereafter culling rate in early lactation could be diminished and health and productive status of cows would be improved. Decreased concentrations of NEFA and  $\beta$ -HBA via drenching treatment, shows improving in NEB and decreasing tissue mobilization and consequently, decreasing in fatty liver and ketosis. Researchers proposed that glucogenic precursors and mineral supplements as a drenching tool, immediately after parturition could be a preventative care and must be part of protocols of fresh cows programs.

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