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Impact of using glucogenic precursors and mineral supplements for prevention of metabolic disorders of Holstein cows at parturition

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Abstract

The objectives of this study were to examine whether drenching or mixed with total mixed ration (TMR) of glucogenic and mineral supplements can prevent metabolic disorders of 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 and for 3 weeks experimental period. The treatments used were: 1) the control treatment that without any addition on water or TMR (CONT), 2) drenching treatment (DRCH) that include: 40 liter water contained 2 kg of the glucogenic and mineral supplements drenched to cows in two equal meals, 3) feeding treatment (FEED) contained two kg of the glucogenic and mineral supplements mixed with TMR, 4) compound diet (DRFD), 20 liter water contained 1 kg of glucogenic and mineral supplements drenched to cows plus 1 kg of these materials mixed in the TMR. DRCH treatment had positive effects on negative energy balance and plasma NEFA and BHBA concentrations. Plasma glucose had increased significantly by treatments ($P<0.05$). Time duration of placenta to fall was significantly lower in DRCH treatment than the other treatments ($P<0.05$). Addition of these glucogenic precursors and minerals on water of fresh cows, resulted in optimize animal metabolic status in postpartum period and reduced duration of falling of placenta. So it can be concluded that drenching of glugenic and mineral supplements at calving improved the cows' health and prevented the metabolic disorders of parturition.

Keywords: Fresh cows, glucogenic precursors, mineral supplements, retained placenta, negative energy balance, blood metabolites

Abbreviation key: CONT = control treatment, **DRCH** = drenching treatment, **FEED** = feeding precursors with TMR, **DRFD** = drenching plus feeding treatment, **NEB** = negative energy balance

Introduction

Transition period in dairy cows is defined as three weeks before calving to three weeks after calving (Grummer, 1995; Goff and Horst, 1997). It was reported that during this period, feeding and management are important factors for the cows. Dry matter intake of lactating cows three weeks before calving will start to fall, because during this period fetal growth would be increased and this adversely affects secretion of hormones (Grummer, 1995). Feed intake may reduce at the amount of 30 percent during the week before calving (Bertics et al., 1992) and cows are often in a negative energy balance at least five weeks after start of lactation (DeFrain et al., 2004). Excessive lipid mobilization leads to increased accumulation of triglycerides in the liver and this cause formation of ketone bodies (Herdt, 1988). Because of inability to overcome the reduced dry matter intake during the transition period, cattle breeders often use drenching and pastes to provide glucose to prevent ketosis and metabolic disorders in pre-partum period (Van Knegsel et al., 2007). Prevention protocols for minimizing negative energy balance, hypocalcemia, ketosis and other diseases have been developed around calving (Goff and Horst, 1997) that simultaneously can help high milk production and reproduction (Miyoshi et al., 2001).

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Drenching of large quantities of water to the cows after calving has two objectives. Firstly, cows lose nearly 60 liters of fluid from the uterus at calving (Doreau et al., 1981), and this causes imbalances in electrolytes, so drenching high volumes of water with electrolytes replaces the lost uterine fluids. Secondly, filling the rumen may lead to rapid move of rumen to the lower abdominal cavity, so it can help to prevent the displacement of the abomasum (Enmark et al., 2009). Propylene glycol is rich of energy (4.7 Mcal NEl/lit) and it could provide the energy requirement of dairy cows in the transition period. Propylene glycol can be easily metabolized and absorbed in the rumen. Fifty percent of propylene glycol is metabolized after 1 to 2 h post feeding and almost 80 to 90 percent of the that remained is metabolized after 3h, transferred to the liver and converted to glucose via gluconeogenesis pathway (Miyoshi et al., 2001). Miyoshi et al. (2001) reported that propylene glycol could stimulate insulin secretion and decrease non esterified fatty acid (NEFA) and ketone bodies in plasma; therefore, it can increase milk production.

Melendez et al. (2002) reported that calcium propionate provides a rapidly absorbable Ca and prevents clinical and subclinical hypocalcemia. They also noted that Ca propionate also minimizes adipose tissue mobilization as a glucogenic precursor; thus, fat deposition in the liver and concentrations of NEFA and beta hydroxyl butyric acid (BHBA) in plasma can be decreased (Christensen et al., 1997) or diminish losses of body condition score (BCS) (Formigoni et al., 1996). Magnesium sulfate provides Mg to maintain natural homeostasis of Ca at parturition, due to its role on secretion of parathyroid hormone (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 it has an important role in acid-base balance and accretion of water in the body and it has a powerful effect on Mg^{2+} absorption (Ammerman, and Goodrich, 1983). K reserves, are limited in the body, and needs to be provided daily (Ammerman and Goodrich, 1983). Choline has three methyle groups and four amine groups those participate in fat transition (Zahra et al., 2006). Choline provides labile methyle groups for transmethylation reactions and it is essential for synthesis of phosphatydile choline of plasma membrane of cells (Zahra et al., 2006). Choline can increase milk fat synthesis and it is 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 synthesis (Drackley et al., 2001).

The objectives of this study were to compare different methods of feeding glucogenic precursors and mineral supplements in early post-parturient period, to 1) alleviate the severity of NEB after parturition, 2) improve metabolic profile of plasma during 21 days of milk, 3) reduce incidence of retained placenta in cows.

Materials and Methods

Cows and experimental design

Immediately after parturition, 72 cows were housed in individual boxes for 24 h. Groups were based on their parity number into three blocks (first lactating block, n=24; second lactating block, n=24; and third lactating block, n=24). Cows of each block were randomly assigned to the four experimental treatments (each had 6 cows). After 24 h, cows were housed at free stalls and received one TMR (table 1) at the rate of 3 times a day (07:30, 13:00, and 18:00 o'clock).

Experimental treatments

The treatments were

1) The control diet without any supplements (CONT)

2) The drenching treatment (DRCH) contains: 2 kg of the glucogenic and mineral supplement dissolved in water and drenched to cows. The cows received these materials with 40 liters of water (at temperature 40˚C) at two times. In the first time, 1 kg of the materials dissolved in 20 liter of water and offered to cows, and in the second time, the other 1 kg of the materials dissolved in 20 liter and was then force fed to cows through esophageal tube.

3) Feeding the glucogenic materials mixed with TMR (FEED): 2 kg of the materials described above were mixed with TMR and fed to cows at three times a day.

4) Compound treatment (DRFD): 1kg of the materials was dissolved in 20 liters of water and 1kg of the materials was mixed with their TMR.

Sample collection

Feed intake and orts were recorded in days 1, 7, 14, and 21 postpartum. Means of DMI intakes in these days were reported as mean of DMI in this trial. 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 at about 4 to 5 hour after morning meal, via tail vein puncture and immediately were centrifuged at 1800×g for 15 min to obtain plasma, which were then stored at 20° c for later analysis, but blood glucose was 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.

Body condition score changes in the beginning and at the end of the experiment were determined on a scale of 1 to 5 scores (Wildman et al., 1982). Time and duration of the placenta falling for each cow were also recorded.

Table 1: Experimental ingredients of diets (%)

Ingredients[1]	Pre-calving	Post-calving
Alfalfa hay	21.744	32.96
Corn silage	16.943	23.60
Wheat straw	0.725	-
Beet Pulp	3.991	4.82
Ground Barley grains	16.240	11.12
Ground Corn grains	9.920	5.76
Whole cottonseed	6.220	3.64
Cottonseed meal	3.240	2.48
Canola meal	2.390	3.65
Soybean meal	7.810	5.07
Fish meal	2.050	1.52
Corn gluten meal	1.610	0.86
Whole soybean grains	2.270	-
Vegetable oil	1.780	1.05
Salt	0.360	-
Sodium bicarbonate	0.880	-
Magnesium oxide	0.420	-
Calcium carbonate	0.360	1.4
Mono calcium phosphate	0.310	-
Vitamin supplement	0.620	0.45
Ammonium sulphate	-	0.44
Calcium chloride	-	0.76
Magnesium sulphate	-	0.43
Total	100	100.01

Containes: 1800000 IU, vit A; 400000 IU, vit D_3; 8000 IU, vit E; 3000 Mg, Antioxidant.

Table2: Chemical composition of experimental diets (on DM basis)

Items	diets	
	Pre-calving	Post-calving
Net Energy for lactation (Mcal/kg)	1.54	1.69
Crude Protein (%)	15.8	17.5
Rumen Degradable Protein (% of CP)	11.5	11.6
Rumen Undegradable Protein (% of CP)	4.3	5.9
Neutral Detergent Fiber (%)	37	32
Forage NDF (%)	27.4	19.2
Acid Detergent Fiber (%)	25	21
Non Fibrous Carbohydrate (%)	36.4	39
Ether Extract (%)	3.8	5.3
Calcium (%)	1.5	1
Phosphorous (%)	0.4	0.5
Dietary Cation Anion Differences (meq/kg)	55	247

Statistical Analysis: The experimental design is a completely randomized block design. All data were analyzed using a MIXED procedure of SAS (2004). Days of blood collection were as repeated measurements. The fixed factors were Treatment (4 treatments), parity order (3 orders) as blocks, and days of sampling relative to calving (1, 7, 14 and 21 after calving). The data were analyzed by 3 way analysis of variance. When treatment effect was significant, comparison between means was done by Tukey test and P<0.05 were declared as significant.

Result and Discussion

Dry matter intake: Least square means of dry matter intake on days 1, 7, 14 and 21 after calving, were 15.46, 18.16, 15.50, and 16.45 kg/d, respectively (table 4). Effect of treatment on dry matter intake were significant (P<0.05). Dry matter intake in DRCH treatment was significantly higher than other treatments (P<0.05). Block (lactation period), had significant effect on dry matter intake (P<0.05) (DMI in first, second and third parities were 14.75, 16.80, and 17.63 kg/d). Interaction between treatment and block also was significant (Table 4).

The potential factor that caused to reduce dry matter intake around calving, was decreased gastrointestinal motility due to hypocalcaemia caused by the increased demand of the fetus and mammary gland to calcium (Goff and Horst, 1997; Marquardt et al., 1977; Ingvartsen, 2006) The most important task of calcium is muscle contraction (Wilde, 2006). Decrease in blood calcium in cows after calving reduced number and strength of rumen contractions that would result in reduced DMI (Daniel, 1983). Use of calcium supplement such as calcium propionate immediately after parturition can be an important factor in improving calcium homeostasis and thus it may improve dry matter intake at calving time. So it could be concluded that one of the reasons for the improved dry matter intake in this study may be due to the improvement of the status of blood calcium by drenching calcium propionate.

In agreement with the results of McNamara and Valdez (2005) who stated that calcium propionate increased dry matter intake by 13% to 11% before and after calving respectively. They reported that cows fed calcium propionate consumed 0.125 kg/d more DM per day. Miyoshi et al. (2001) found that feeding propylene glycol in the transition period significantly increased dry matter intake. In the study of Zahra et al. (2006), dry matter intake increased by feeding of 14 g rumen protected choline for three weeks before calving to 4 weeks after calving in obese cows (body condition score at 3 weeks before parturition is more than 4) but such response was not observed in non-obese cows. Abomasal infusion of 90 g per day choline caused more quadratic effect on dry matter intake than infusion of30 grams of choline (Sharma and Erdman, 1989). Patton et al. (2004) reported that feeding a mixture of propylene glycol with calcium propionate maintained dry matter intake to 16 kg per day at calving day (interaction between treatment and day, (P≤0.05) (Table 5).

In the study of Bryant et al. (1999), daily dry matter intake with the addition of rumen protected choline was higher than controls. Bindel et al. (1998) reported that average daily gain and dry matter intake and feed efficiency for heifers receiving 10 g of not

encapsulated choline chloride was higher than for the control group. Chung et al. (2009) also reported that the addition of rumen protected choline to the diet increased the amount of dry matter intake. They noted that choline improved appetite. Perhaps this response is due to the ability of choline to improve liver function (Piepenbrink and Overton, 2000; Cooke et al., 2007). Unlike the results of this study, de Ondarza et al. (2007) found a significant decrease in dry matter intake in a commercial herd that fed 3.1 gram of rumen protected choline for a period more than 4 weeks. Feeding of 282 g/d of rumen unprotected choline, decreased dry matter intake, while 159 grams of unprotected choline had no effect on dry matter intake (Sharma and Erdman, 1989). Inconsistent to this study, the above results may be related to the number of days of measuring the DMI, the treatment method, amount and frequency of feeding of glucogenic precursors and mineral supplements.

Unlike the results of this study in drench treatment, McFadden et al. (2010) reported that the average dry matter intake during the first 10 days of lactation was not affected by drenching treatment. They drenched in 3 treatments to cows in the first 24 hours after parturition. Their treatments included 37.85 liters of water (control), 37.85 liters of water plus 355 g of propylene glycol (PG) and 37.85 liters of water plus 900 g of a commercial product (DDP), which contains equal amounts of glucose precursor that was equal with PG treatment. . Fisher et al. (1973) reported a 9% reduction in dry matter intake with feeding of 9% propylene glycol mixed with concentrate. Chung et al. (2009) also reported propylene glycol had no effect on dry matter intake.

DeFrain et al. (2004) reported that feeding propionate did not affect dry matter intake in the first 21d postpartum. Similarly, Chung et al. (2009) reported that feeding propylene glycol in combination with TMR or top dressing, did not affect dry matter intake.

Body condition score changes: Body condition score changes in the DRCH treatment were insignificantly (P = 0.09) lower than the other treatments (Table 4). Block had significant effect on changes in body condition score (P<0.05) but the interaction between treatment and block was not significant (P>0.05).

Obese cows at calving have incidence to milk fever 4 times more than normal ones (Ostergaard et al., 2003). Hypocalcaemia is likely high in fat cows that trigger immune system suppression and may cause dystocia and retained placenta (Houe et al., 2001).

In agreement with the results of this study, McNamara and Valdez (2005) reported that calcium propionate had no effect on body weight (BW) and body condition score. Pickett et al. (2003) did not report any effect of propylene glycol on body weight and body condition score within three weeks of lactation. With respect to results obtained for serum NEFA concentrations, the lowest concentration was observed for drenching treatment that also had a low body condition score change. It could be concluded that the use of glucogenic materials and mineral supplements are effective factors in improving the negative energy balance after calving.

Time duration of placenta fall in control, drenching, feeding and combined treatments were 7.86, 4.55, 7.63, and 9 hour, respectively. The effect of treatment was significant. Comparison of the means by Tukey test showed that the time duration for falling of the placenta of drenching treatment was significantly more than that for the other treatments (Table 4). Kimura et al. (2000) suggested that retained placenta, partly related to reduction of the power of the immune system to degeneration of fetus placentum. Reduction of the power of the immune system at pre-partum period is multifactorial, but is related to endocrine changes and reduced dry matter intake and decreased nutrient intake

Table 3: Least square means of plasma metabolits[1]

	Treatments					P-Value[3]			
Items	CONT[1]	DRCH	FEED	DRFD	SEM[2]	T	B	D	B×T
Glucose(mg/dl)	58.77[b]	64.48[a]	56.35[b]	55.71[b]	1.53	0.0001	0.0001	0.0001	0.166
NEFA(mmol/l)	0.62[c]	0.53[b]	0.79[a]	0.68[ac]	0.035	0.0001	0.0001	0.0001	0.0001
BHBA(mmol/l)	0.59[a]	0.51[b]	0.51[c]	0.53[b]	0.021	0.0001	0.0001	0.0706	0.0001

CONT = control treatment, **DRCH** = drenching treatment, **FEED** = feeding precursors with TMR, **DRFD** = drenching plus feeding treatment; Standard error of mean; T = treatment effect, B = block effect (parity number), D = day effect (day of sample collection)

Table 4: Least square means of DMI, BCS changes, and duration of placenta falling[1]

	Treatments					P-Value[3]			
Items	CONT[1]	DRCH	FEED	DRFD	SEM[2]	T	B	D	B×T
DMI (kg/d)	15.46[b]	18.16[a]	15.50[b]	16.45[c]	0.31	0.0001	0.0001	0.0001	0.0108
BCS changes[4]	-0.36	-0.28	-0.38	-0.39	0.035	0.09	0.003	-	0.66
Duration of placenta falling (h)	7.86[a]	4.55[b]	7.63[ab]	9.0[a]	1.18	0.042	0.0008	-	0.20

CONT = control treatment, **DRCH** = drenching treatment, **FEED** = feeding precursers with TMR, **DRFD** = drenching plus feeding treatment; Standard error mean; T = treatment, B = block, D = day; Based on 1-5 scale (1= thin, 5= obese)

(Goff and Horst, 1997). Melendez et al. (2004) reported that the plasma calcium concentration in the cows with retained placenta is lower than healthy cows, of course, not in cows with milk fever but in hypocalcaemic cows. In agreement with the findings of this research, Oetzel et al. (1996) reported that calcium supplementation did not have significant effect on the incidence of retained placenta, but the tendency to decrease was observed in treated than control. Melendez et al. (2003) also reported that feeding calcium supplements had no significant effect on the incidence of retained placenta.

Plasma metabolites

Glucose: Least square means of plasma glucose of treatments were 58.77, 64.48, 56.35 and 55.71 mg/dl, respectively (Table 3). There were significant differences between treatments ($P<0.05$). This increase, would be related to improvement in DMI and energy status of cows (Bertics et al., 1992).

In agreement with the current findings, Lien et al. (2010) suggested propylene glycol that drenched one time a day to result in the increase of plasma glucose. Miyoshi et al. (2001) found that feeding propylene glycol in transition period significantly increased plasma glucose. Propylene glycol converted to propionic acid in the rumen (Grummer et al., 1994), and then converted to glucose in the gluconeogenesis pathway. In agreement with the present findings in FEED treatment, Chung et al. (2009) and Castaoeda-Gutiérrez et al. (2009) did notreport any effects of feeding propylene glycol with TMR on plasma glucose. McNamara and Valdez (2005) did not show any effects of feeding Ca propionate on plasma glucose.

NEFA and BHBA: The least square means of non esterified fatty acids and beta hydroxyl butyric acid between treatments were: 0.62, 0.53, 0.79, 0.68 mmol/l and 0.59, 0.51, 0.51 and 0.53 mmol/l, respectively. Mc Fadden et al. (2010) reported that drenching propylene glycol or a package contained glucogenic precursors plus minerals decreased NEFA and BHBA. Enmark et al. (2009) suggested that drenching Ca propionate, potassium chloride, and magnesium sulfate in 20 liter water had significant effects on NEB and decreased the concentrations of BHBA and NEFA. Since, choline is a lipotropic factor, it seems to have beneficial effects on adipose tissue and liver metabolism (Piepenbrink and Overton, 2000). Significant effects of treatment and time interaction were observed on plasma concentration of NEFA and ratios of NEFA to cholesterol. This indicated that the increase of NEFA in cows supplemented with protected choline was lower than in the 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 BHBA in cows received choline, compared to CONT treatment, was due to the role of the supplied choline in fatty acid metabolism (Pinotti et al., 2003). The current findings were in agreement with Piepenbrink and Overton (2000) who reported that protected choline, in pre-parturient period, could decrease deposition of FA in the liver and increased amounts of glycogen.

Conclusion

The 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 alone or with drenching. These results would be related to decreased incidence of hypocalcemia, milk fever, hypomagnesemia, retained placenta and displaced abomasum and thereafter, culling rate in early lactation could be diminish and health and productive status of cows would be improved. Decreased concentrations of NEFA and BHBA via drenching treatment, showed improvment in NEB and decreasing tissue mobilization and consequently, decreasing in fatty liver and ketosis.

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REFERENCES

Ammerman, C.B. and Goodrich R.D. 1983. Advances in mineral nutrition in ruminants. *Journal of Animal Science,* 57 suppl. 2:519–533

Bertics, S.J., Grummer, R.R., Cadorniga-Valino, C., and Stoddard E.E. 1992. Effect of prepartum dry matter intake on liver triglyceride concentration and early lactation. *Journal of Dairy Science,* 75:191-192.

Bindel, D.J., Drouillard, J.S., Titgemeyer, E.C., Wessels, R.H. and Loest, C.A. 1998. Effects of ruminally protected choline and dietary fat on performance of finishing heifers. *Journal of Animal Science,* 76:321-335.

Bryant, T.C., Rivera, J.D., Galyean, M.L., Duff, G.C., Hallford, D.M. and Montgomery, T.H. 1999. Effects of dietary level of ruminally protected choline on performance and carcass characteristics of finishing beef steers and on growth and serum metabolites in lambs. *Journal of Animal Science,* 77:2893-2903.

Castaoeda-Gutiérrez, E., Pelton, S H., Gilbert, R.O. and Butler, W.R. 2009. Effect of peripartum dietary energy supplementation of dairy cows on metabolites, liver function and reproductive variables. *Animal Reproduction Science,* 112:301.

Christensen, J.O., Grummer, R.R., Rasmussen, F.E., and Bertics, S.J. 1997. Effect of method of delivery

of propylene glycol on plasma metabolites of feed-restricted cattle. *Journal of Dairy Science,* 80:563-572.

Chung, Y.H., Brown, N.E., Martinez, C.M., Cassidy, T.W. and Varga, G.A. 2009. Effects of rumen-protected choline and dry propylene glycol on feed intake and blood parameters for Holstein cows in early lactation. *Journal of Dairy Science,* 92: 2729-2735.

Cooke, R.F., Silva del Rio, N., Caraviello, D.Z., Bertics, S.J., Ramos, M.H. and Grummer, R.R. 2007. Supplemental choline for prevention and alleviation of fatty liver in dairy cattle. *Journal of Dairy Science,* 90:2413–2418.

Daniel, R.C.W. 1983. Motility of the rumen abomasum during hypocalcemia. *Canadian Journal of Comparative Medicine,* 47:276-280.

de Ondarza, M. B., Emanuele, S. and Putnam, D. 2007. Effect of rumen protected choline (Reashure) supplemented to highproducing cows on milk production, milk components, and intake. *Journal of Dairy Science,* 90 (Suppl. 1): 353. (Abst)

DeFrain J.M., Hippen, A.R., Kalscheur, K.F. and Jardon, P.W. 2004. Feeding glycerol to transition dairy cows: effects on blood metabolites and lactation performance. *Journal of Dairy Science,* 87: 4195–4206.

Doreau, M., Journet, M., Marquis, B., Ollier, A., Flechet, J., LeFaivre, R. and Sornet R. 1981. Variations de la digestibilité de la ration et de quelques paramétres sanguint chez la vache laitiére á proximité do vélage. Bulletine Techn C.R.Z.V. *Theix, INRA,* 44: 17–22.

Drackley, J.K., Oveton, T.R. and Douglas, G.N. 2001. Adaptation of glucose and long chain fatty acid metabolis in liver of dairy cows during the periparturient period. *Journal of Dairy Science,* 84(suppl.):E100-112.

Enemark, J.M.D., Schmidt, H.B., Jakobsen, J. and Enevoldsen, C. 2009. Failure to improve energy balance or dehydration by drenching transition cows with water and electrolytes at calving. *Veterinary Research Communication,* 33:123–137.

Fisher, L.J., Erfle, J.D., Lodge, G.A. and Sauer, F.D. 1973. Effects of propylene glycol or glycerol supplementation of the diet of dairy cows on feed intake, milk yield and composition, and incidence of ketosis. *Canadian Journal of Animal Science,* 53:289.

Formigoni, A., Cornil, M., Prandi, A., Mordenti, A., Rossi, A., Portetelle, D. and Renaville, R. 1996. Effect of propylene glycol supplementation around parturition on milk yield, reproduction performance and some hormonal and metabolic characteristics in dairy cows. *Journal of Dairy Research,* 63: 11–24

Goff, J.P. and Horst, R.L. 1997. Physiological changes at parturition and their relationship to metabolic disorders. *Journal of Dairy Science,* 80:1260–1268.

Grummer, R.R. 1995. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. *Journal of Animal Science,* 73: 2820- 2833.

Grummer, R.R., Winkler, J.C., Bertics, S.J. and Studer, V.A. 1994. Effect of propylene glycol dosage during feed restriction on metabolites in blood of prepartum Holstein heifers. *Journal of Dairy Science,* 77: 3618-3623.

Herdt, T.H. 1988. Fatty liver in dairy cows. *The Veterinary Clinics of North America. Food Animal Practice 4*, 269–287.

Houe, H., Ostergaard, S., Thilsing-Hansen, T., Jorgensen, R.J., Larsen, T., Sorensen, J.T., Agger, J.F. and Blom, J.Y. 2001. Milk fever and subclinical hypocalcaemia–an evaluation of parameters on incidence risk, diagnosis, risk factors and biological e.ects as input for a decision support system for disease control. *Acta Veterinaria Scandinavica,* 42:1–29.

Ingvartsen, K.L. 2006. Feeding- and management-related diseases in the transition cow Physiological adaptations around calving and strategies to reduce feeding-related diseases. *Animal Feed Science and Technology,* 126:175–213.

Kimura, K., Goff, J. and Tyler, H. 2000. Association between retained placenta and neutrophil function in dairy cattle. *Journal of Dairy Science,* 83(suppl.1):3 (Abst.).

Lien, T.F., Chang, L.B., Horng, Y.M. and Wu, C.P. 2010. Effects of propylene glycol on milk production, serum metabolites and reproductive performance during the transition period of dairy cows. Asian-Aust. *Journal of Animal Science,* 23(3):372-378.

Marquardt, J.P., Horst, R.L. and Jorgensen, N.A. 1977. Effect of Parity on Dry Matter Intake at Parturition in Dairy Cattle1. *Journal of Dairy Science,* 60: 929-934.

McFadden, J.W, Wallace, R.L. and Drackley, J.K. 2010. Effects of a drinkable drench in primiparous Holstein cows. *The Professional Animal Scientist,* 26:159–166.

McNamara, J.P. and Valdez, F. 2005. Adipose tissue metabolism and production responses to calcium propionate and chromium propionate. *Journal of Dairy Science,* 88:2498-2507.

Melendez . P., Donovan, A., Risco, C.A., Hall, M.B., Littell, R. and Goff, J.P. 2002. Metabolic responses of transition Holstein cows fed anionic salts and supplemented at calving with calcium and energy. *Journal of Dairy Science,* 85:1085–1092.

Melendez, P., Donovan, G.A., Risco, C.A., Littell, R. and Goff, J.P. 2003. Effect of calcium-energy supplements on calving-related disorders, fertility and milk yield during the transition period in cows fed anionic diets. *Theriogenology,* 60: 843–854

Melendez, P., McHale, J., Bartolome, J., Archbald, L.F. and Donovan, G. A. 2004. Uterine involution and fertility of Holstein cows subsequent to early postpartum PGF2α treatment for acute puerperal metritis. *Journal of Dairy Science,* 87:3238–3246.

Miyoshi, S., Pate, J.A. and Palmquist, D.A. 2001. Effects of propylene glycol drenching on energy balance, plasma glucose, plasma insulin, ovarian function and conception in dairy cows. *Animal Reproduction Science,* 68:29-43.

Oetzel, G.R. 1996. Improving reproductive performance in dairy cattle via milk fever prevention. The Bovine Proceedings. 28:52-59

Ostergaard, S., Sorensen, J.T. and Houe, H. 2003. A stochastic model simulating milk fever in a dairy herd. *Preventive Veterinary Medicine,* 58:125-143.

Patton, R.S., Sorenson, C.E. and Hippen, A.R. 2004. Effects of dietary glucogenic precursors and fat on feed intake and carbohyrate status of transition dairy cows. *Journal of Dairy Science,* 87: 2122-2129.

Pickett, M.M., Piepenbrink, M.S. and Overton, T.R. 2003. Effects of propylene glycol or fat drench on plasma metabolites, liver composition, and production of dairy cows during the periparturient period. *Journal of Dairy Science,* 86, 2113–2121.

Piepenbrink, M.S. and Overton, T.R. 2000. Liver metabolism and production of periparturient dairy cattle fed rumen-protected choline. *Journal of Dairy Science,* 83 (Suppl. 1): 257 (Abst.).

Pinotti, L., Baldi, A., Politis, I., Rebucci, R., Sangalli, L. and Dell'Orto, V. 2003. Rumen protected choline administration to transition cows: Effects on milk production and vitamin E status. *Journal of Veterinary Medicine,* A 50: 18–21.

SAS Institute. 2004. SAS/STAT® User's Guide, Version 9.1 Edition. SAS Inst., Inc., Cary, NC.

Sharma, S. and Erdman, R.A. 1989. Effects of dietary and abomasally infused choline on milk production responses of lactating dairy cows. *Journal of Nutrition,* 119:248–254.

Van Knegsel, A.T.M., Van Den Brand, H., Dijkstra, J., Van Straalen, W.M., Jorritsma. R., Tamminga, S. and Kemp, B. 2007. Effect of glucogenic vs. lipogenic diets on energy balance, blood metabolites, and reproduction in primiparous and multiparous dairy cows in early lactation. *Journal of Dairy Science,* 90: 3397–3409.

Wilde, D. 2006. Influence of macro and micro minerals in the periparturient period on fertility in dairy cattle. *Animal Reproduction Science,* 96:240-249.

Wildman, E.E., Jones, G.M., Wagner, P.E., Boman, R.L., Troutt, H.F. and Lesch, T.N. 1982. A dairy cow body condition scoring system and its relationship to standard production characteristics. *Journal of Dairy Science,* 65:495-501.

Zahra, L.C., Duffield, T.F., Leslie, K.E., Overton, T.R., Putnam, D. and LeBlanc, S.J. 2006. Effects of rumen-protected choline and monensin on milk production and metabolism of periparturient dairy cows. *Journal of Dairy Science,* 89:4808–4818.