Iranian Journal of ruminants Health Research, 2016, 1 (2), 1-8
Doi: 10.22055/ijrhr.2017.12841

# The Degrees of Parturient Hypocalcemia and Its Relevance to Other Metabolic Profile Parameters in Downer Cows

***Kojouri, G.A[1*].; Hedayati. F.S[2].; Zandi, S[2]., Karimzadeh. J[3].; Taheri, F[3].; Ansari, F[3].; Yazdani, M[3].; Davoodi, Z[2], Mostafavi, M[2].; Eshraghi Samani, R[4]***

*[1] Department of Clinical Science, School of Veterinary Medicine, Shahrekord University, Shahrekord, Iran.*

*[2] DVM, Graduated student of School of Veterinary Medicine, Shahrekord University, Shahrekord, Iran.*

*[3] DVM, Veterinarians working in private sector.*

*[4] Student of Veterinary Medicine, School of Veterinary Medicine, Shahrekord University, Shahrekord, Iran.*

*****Corresponding author:*** *drgholam_alikojouri@yahoo.com; kojouri@vet.sku.ac.ir*

## Abstract

**At calving time, the cows were monitored and blood samples were taken from jugular vein of 38 cows with parturient paresis and 38 healthy ones. The levels of calcium, phosphorus, magnesium, sodium, potassium, chloride, glucose, triglyceride, cholesterol, albumin, globulin and A/G ratio were assessed to find the correlation between the degree of hypocalcemia and other parameters. According to calcium concentration of milk fever cows (downers), five subgroups (<5 mg/dl as a); 5.1-6 as b); 6.1-7 as c); 7.1- 8 as d) and 8.1< as e)) were made and parameters were analyzed statistically as well. Results indicated that serum levels of calcium and phosphorus in downers were lower and magnesium concentration was significantly higher than that of the control group (P<0.05). A significant relationship was observed between level of daily phosphorus intake and $^{k}/_{Ca+Mg}$ ratio and the incidence of milk fever (p<0.05). Calcium and glucose concentration of subgroup a) was significantly lower and higher than the other subgroups. Potassium was negatively correlated with calcium in subgroups b) and d). Potassium was also negatively and positively correlated with glucose and cholesterol in subgroup b). But in subgroups d) and e), potassium was positively correlated with chloride and glucose. So, the researchers of the present study concluded that, hypophosphatemia is a major cause of prolonged sternal recumbency and poor response to routine therapy for milk fever. In addition, negative correlation between calcium and potassium concentration and also the presence of hyperglycemia in severe hypocalcemia (subgroup a)) may intervene with successful treatment as well .**

**Keywords**: Parturient paresis, Milk fever, Parturient hypocalcemia, Hypophosphatemia, Dairy cattle.

## Introduction

One of the most important disorders in veterinary science and particularly dairy cattle is metabolic or production diseases. Milk fever, ketosis, downer cow syndrome, post parturient hemoglubinuria, hypomagnesemia and hypokalemia are the examples of such diseases. Milk fever (Parturient paresis, Parturient hypocalcemia, Paresis puerperalis and Parturient apoplexy), occurring around the time of parturition, is characterized by progressive neuromuscular dysfunction with flaccid paralysis, circulatory collapse, depression of consciousness and ultimately shock and death (Radostits et al., 2007). The main cause of the disease is a depression of ionized calcium in serum and tissues. Hypophosphatemia and variations in levels of serum magnesium also occur in milk fever and have secondary roles like type II ketosis (high blood insulin and transient hyperglycemia) which is seen immediately after calving (Smith, 2015).

The transition period consists of two phases, the first being formed by the last three weeks before calving and the second by the first three weeks of postpartum. This period is marked by changes some of which are related to alterations increases in energy requirements driven by both fetal needs and lactogenesis, endocrine and metabolic preparing cows for childbirth and lactation (Morgante et al., 2012). During the last 2 weeks pre-calving, dairy cows are usually in negative energy balance and calcium and in the last days before calving, the balance of other nutrients such as protein, vitamins and minerals may also be compromised. This reduction in serum calcium concentrations usually occurs about 12 to 24 hours after calving (Kimura et al., 2006; Goff, 2008). The level of calcium in plasma is well regulated, and when the level decreases, the parathyroid gland will excrete parathyroid hormone (PTH) (Oetzel & Miller, 2012). Increasing in nonesterified fatty acid and reduction of free cholesterol, cholesteryl ester and phospholipid concentrations in cows with milk fever were observed by Yamamoto et al (2001), but the degrees of hypocalcemia and its effect on biochemical parameters have not been studied yet. For these reasons, the purpose of the present study was to examine the changes of biochemical and mineral status in milk fever cows and to determine the relevance between the degrees of hypocalcemia and other parameters as well.

## Material and Methods

The present research was conducted on forty industrial dairy farms which contain 1625 heads of dairy cattle in Shahrekord district (IRAN).

### *Ration analysis*

Samples of dry period diet were taken and milk fever occurrence was assessed in a prospective study. Level of phosphorus, calcium, magnesium and the ratio of $^{Ca}/_{p}$ and $^{k}/_{Ca+Mg}$ were determined. Total ration was collected and sent to the laboratory for determining the crude protein (CP) and dry matter (Goldberg et al, 1992) as well as the Ca, P, Mg, K, Cl, Na, and sulphate contents. The CP analysis was performed according to the macro-Kjeldahl method as described earlier and calculated as percentage of N × 6.25 (Conklin-Brittain et al., 2004). Na, K, Ca, and Mg were measured by atomic absorption spectrophotometry (model no. 3030, Perkin-Elmer, Norwalk, CT) following acid digestion. Phosphorus was determined colorimetrically using a Beckman DU-60 spectrophotometer (Beckman Instruments, Inc., Fullerton, CA) following acid digestion. Chloride was extracted using a combination of acetic acid and nitric acid and was determined by chloridometer (Haake Buchler Instruments, Inc., Saddle Brook) (Chan et al., 2006).

### *Biochemical analysis*

At calving time, the cows were monitored and blood samples were taken from jugular vein of 38 cows with parturient paresis and 38 healthy ones.

The serum total protein concentrations were determined in the samples using an auto analyzer utilizing a biuret reaction as described earlier in this paper (Burtis and Ashwood, 1994). After dilution, sera were analyzed for Na, K, and Ca using an atomic absorption spectrophotometer (Shimadzu AA, 670, Japan). Serum Cl concentration was determined using an auto analyzer according to the method described by Randall et al. (1974). Serum phosphorus and magnesium concentrations were measured by colorimetric and flame photometric methods, respectively (Burtis and Ashwood, 1994). Triglyceride and cholesterol concentrations of the serum samples were measured using an autoanalyzer (Perkin-Elmer, USA) and optical densities were measured at 340-680 nm (Burtis and Ashwood, 1994).

According to the calcium concentration of milk fever cows, five subgroups (<5 mg/dl as a); 5.1-6 as b); 6.1-7 as c); 7.1- 8 as d) and 8.1< as e)) were made and parameters were analyzed statistically (Table 1).

**Table 1:** Frequencies of calcium related subgroups

| Subgroups | serum calcium centration (mg/dl) | Frequency |
|---|---|---|
| **a)** | <5 | 5 |
| **b)** | 5.1 to 6 | 15 |
| **c)** | 6.1 -7 | 11 |
| **d)** | 7.1-8 | 4 |
| **e)** | 8.1< | 3 |

### Statistical analysis

The data were studied statistically by One Way Analysis of Variance (ANOVA), Chi-Square and Pearson correlation analysis, at the level of $P<0.05$ by SPSS program.

### Results

Dry period ration crude protein (CP), dry matter, Ca, P, Mg, K, Cl, Na, and sulphate ($HSO_4^-$), in healthy cows, were 14.32 ± 1.23%, 19.52 ± 1.48%, 0.776 ± 0.041%, 0.268 ± 0.022%, 0.51 ± 0.037%, 0.8 ± 0.055%,0.71 ± 0.032%, 0.135 ± 0.021% and 0.71 ± 0.065%, respectively. Statistical analysis showed that, in cows with milk fever, the dry matter (17.24 ± 2.01%), Cl (0.61 ± 0.05%) and Na (0.088 ± 0.01%) concentration of dry period rations were significantly lower compared to the healthy ones ($P<0.05$).

Results also indicated that the incidence of milk fever was approximately 2.34% and there was no significant relationship between levels of dietary calcium and magnesium intake and the ratio of $^{Ca}/_{p}$ and incidence of milk fever. But a significant relationship was observed between level of daily phosphorus intake (more than 80 grams per day) and ratio of $^{k}/_{Ca+Mg}$ (less than 1) and the incidence of milk fever ($p<0.05$).

The comparison between serum calcium, phosphorus, magnesium, sodium, potassium, chloride, glucose, triglyceride, cholesterol, albumin and globulin concentration in both cows with milk fever and healthy ones is shown in table 2. The obtained results indicated that cows with milk fever suffered from low level of serum calcium, phosphorus, triglyceride, cholesterol, total protein and globulin and, conversely, high serum level of magnesium and glucose compared to the control group ($P<0.05$). The findings also showed that albumin concentration and albumin/globulin (A/G) ratio significantly decrease if the cows lay down for more than 6 hours.

In cows with milk fever, many significant correlations were observed between parameters (Table 3). In subgroup $e_{)}$ ***calcium*** was negatively correlated with albumin (P<0000001) and positively with phosphorus ($P=0.0293$). ***Phosphorus*** was positively correlated with glucose ($a_{)}$), cholesterol ($a_{)}$) and sodium ($d_{)}$) and negatively with albumin ($e_{)}$) ($P<0.05$). ***Potassium*** was negatively correlated with calcium ($b_{)}$ and $d_{)}$), glucose ($b_{)}$) and total protein ($d_{)}$) and positively with cholesterol ($b_{)}$), chloride ($d_{)}$) and glucose ($e_{)}$) ($P<0.05$). ***Triglyceride*** was negatively correlated with total protein and albumin ($a_{)}$) and positively correlated with cholesterol ($b_{)}$), chloride ($d_{)}$) and glucose ($e_{)}$) ($P<0.05$).

**Discussion**

Milk fever is a metabolic disorder of calcium homeostasis that affects about 2% to 6% of postpartum cows (Anderson and Rings, 2009; Smith 2015). Due to the obtained results, the incidence of milk fever was estimated as 2.34% in Shahrekord district.

Calcium is tightly regulated in mammals because of the critical role of calcium ion concentrations in many physiological functions. Sudden calcium outflow occurs most commonly at the time of the initiation of lactation. The calcium demand associated with colostral production in dairy cows exceeds the total prepartum calcium requirements (mineralization of fetal skeleton). Dairy cows producing colostrum (containing 1.7 to 2.3 g of Ca per kilogram) or milk (containing 1.2 g of Ca per kilogram) typically secrete 20 to 30 g of Ca each day in early lactation (Smith, 2015). Less severe disturbances in blood Ca concentration cause reduced feed intake, poor rumen, uterine and intestine motility and increase susceptibility to other metabolic and infectious disease (Goff, 2008). According to the results of the present

***Table 2:*** *Comparison between serum biochemical and mineral concentration of milk fever cows*

| | **Ca (mg/dl)** | **P (mg/dl)** | **Mg (mg/dl)** | **K (mEq/L)** | **Na (mEq/L)** | **Cl (mEq/L)** | **Glucose (mg/dl)** | **Triglyceride (mg/dl)** | **Cholesterol (mg/dl)** | **Total protein (g/dl)** | **Albumin (g/dl)** | **Globulin (g/dl)** | **A/G ratio** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Control** | 9.52±0.48 | 6.32±0.74 | 1.23±0.38 | 4.43±0.22 | 142.46±1.19 | 121.46±8.67 | 63.1±13.28 | 23.63±6.84 | 185.26±60.06 | 8.04±1.34 | 3.68±0.42 | 4.35±1.47 | 1.01±0.63 |
| **Downers** | 6.38±0.05 | 2.51±1.44 | 2.84±0.40 | 4.36±0.86 | 144.34±5.49 | 121.08±11.64 | 88.2±28.2 | 15.91±6.72 | 110.97±46.96 | 6.92±0.77 | 3.62±0.37 | 3.29±0.79 | 1.19±0.45 |
| **P value** | **P<0.05** | **P<0.05** | **P<0.05** | NS | NS | NS | **P<0.05** | **P<0.05** | **P<0.05** | **P<0.05** | NS | **P<0.05** | NS |

***Table 3:*** *Calcium dependent serum biochemical and mineral concentration in milk fever cows*

| **Subgroups of milk fever cows** | **Ca (mg/dl)** | **P (mg/dl)** | **Mg (mg/dl)** | **K (mEq/L)** | **Na (mEq/L)** | **Cl (mEq/L)** | **Glucose (mg/dl)** | **Triglyceride (mg/dl)** | **Cholesterol (mg/dl)** | **Total protein (g/dl)** | **Albumin (g/dl)** | **Globulin (g/dl)** | **A/G ratio** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **a)** | 4.66±0.09 | 2.5±0.44 | 2.99±0.35 | 4.62±0.2 | 148.1±0.71 | 122.9±3.42 | 104.1±3.1 | 15±0.7 | 93.6±6.69 | 7.05±0.11 | 3.47±0.08 | 3.58±0.04 | 0.97±0.03 |
| **b)** | 5.38±0.03 A | 2.38±0.27 | 2.82±0.14 | 4.24±0.16 | 145.46±0.83 | 119.9±2.61 | 79.73±4.93 A | 14.86±0.86 | 99.26±5.87 | 6.88±12 | 3.6±0.09 | 3.28±0.11 | 1.12±0.06 |
| **c)** | 6.7±0.02 AB | 2.69±0.44 | 2.71±0.19 | 4.39±0.36 | 142.7±2.48 | 124.8±4.48 | 90.63±7.78 A | 19.18±3.24 | 112.54±18.37 | 7.06±0.18 | 3.65±0.13 | 3.41±0.22 | 1.12±0.1 |
| **d)** | 7.25±0.02 ABC | 1.37±0.4 | 2.87±0.59 | 4.47±0.09 | 144.5±2.6 | 119±2.85 | 76.33±4.5 A | 13.75±1.25 | 99±11.56 | 6.52±0.18 A | 3.57±0.2 | 2.95±0.24 A | 1.24±0.16 |
| **e)** | 8.13±0.12 ABCD | 3.33±1.21 | 2.79±0.19 | 4.08±0.17 | 141±1 AB | 115±6.42 | 76±7.5 A | 12.66±1.45 | 165.66±39.81 AB | 8.32±0.31 ABCD | 3.76±0.12 | 4.56±0.23 ABCD | 0.82±0.04 A |

A: *Significant to subgroup a)*

B: *Significant to subgroup b)*

C: *Significant to subgroup c)*

D: *Significant to subgroup d)*

study, twenty-one percent of milk fever cows were suffered from retained placenta and fifteen percent from dystocia.

In the current study, significantly lower serum Ca and P concentrations with higher level of Mg were recorded in milk fever cows (subgroup $a_{)}$) and, in line with our findings, Larsen et al. (2001) suggest that rectal temperature, inorganic blood phosphate, and potassium of milk fever cows, were directly correlated with decline blood calcium, while glucose, lactate, and magnesium were inversely associated with calcium.

Fenwick (1988) postulated that there were significant differences in the concentrations of serum magnesium and plasma sodium concentrations for normal VS. milk fever cows, respectively ($P<0.05$). Serum sodium concentration in subgroup $e_{)}$ was significantly lower than that subgroups $a_{)}$ and $b_{)}$ ($P<0.05$).

According to the results and on the basis of increasing calcium level (subgroups $a_{)}$ to $e_{)}$), the glucose concentration inversely decreased significantly ($P<0.05$). In other words, the cows with lower calcium level suffered from hyperglycemia. Similarly, in subgroup $e_{)}$ the decline in serum sodium was significantly correlated inversely with calcium concentration.

Moreover, the results indicated that the duration of dry period was negatively correlated with the glucose concentration in cows with milk fever. It means that cows with shorter dry off period (one month) have higher glucose level if lying down.

Smith (2015) stated that ruminants are considered to be relatively insulin resistant but, during early lactation, low insulin concentrations are accompanied by high tissue insulin sensitivity. However, Anderson and Rings (2009) reviled that as cows approach calving, the sensitivity of tissues to insulin is normally reduced, apparently in association with hormonal changes associated with impending parturition. As previously mentioned, within the periparturient period, the dry matter of milk fever cows ration was lower than the healthy ones and it may cause loss of hepatic function. In this regard, Anderson and Rings (2009) explain that a reduction in feed intake (dry matter intake or DMI) occurs in almost all cows as they approach parturition, but it is exacerbated in obese cows and those under conditions of environmental and nutritional stress. In some cows, this leads to negative energy balance before calving, and in nearly all cows energy is in negative balance in early lactation as the postpartum increase in DMI fails to keep pace with increased energy demands of milk production.

In this way, it was reported that the amount of hepatic lipids was increased whereas the hepatic output pathways were reduced leading to decreases in the circulating concentrations of total lipids, triglycerides, cholesterol and lipoproteins (Bobe et al., 2004; Radostits et al., 2007). Accordingly, the observed decreases in the serum triglyceride and cholesterol concentrations would result from a transient impairment of the liver function during the periparturient period.

Increase in nonesterified fatty acid and reduction of free cholesterol, cholesteryl ester and phospholipid concentrations in cows with milk fever, were observed by Yamamoto et al. Our findings stated that in cows with milk fever, dystocia could alter serum cholesterol concentration and significantly elevated its level compared to the downers with normal parturition ($P<0.05$). Furthermore, cholesterol, total protein and globulin concentrations of subgroup e) were significantly higher than those in subgroups a) and b) ($P<0.05$), suggesting the role of other factors in laying down the cows with parturient hypocalcemia.

According to the obtained results, the researchers concluded that hypophosphatemia is a major cause of prolonged sternal recumbency and a poor response to routine therapy for milk fever. In addition, negative correlation between calcium and potassium concentration and also the presence of hyperglycemia in severe hypocalcemia (subgroup a)) may intervene with successful treatment as well .

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# تأثیر درجات مختلف هیپوکلسمی زایمانی بر دیگر فراسنجه های متابولیکی در گاوان زمین گیر

غلامعلی کجوری[1]*، فاطمه سادات هدایتی[2]، شیرین زندی[2]، جهانبین کریمزاده[3]، فریدون انصاری[3]، فرهاد طاهری[3]، محسن یزدانی[3]، زینت داوودی[2]، مریم مصطفوی[2]، راضیه اشراقی سامانی[4]

۱- گروه علوم درمانگاهی، دانشکده دامپزشکی، دانشگاه شهرکرد، ایران.

۲- دانش آموخته دانشکده دامپزشکی، دانشگاه شهرکرد، ایران.

۳- دامپزشکان شاغل در بخش خصوصی.

٤- دانشجوی دکتری دامپزشکی، دانشکده دامپزشکی، دانشگاه شهرکرد، ایران.

*نویسنده مسئول: drgholam_alikojouri@yahoo.com

**خلاصه:**

**پس از زایمان وقوع هیپوکلسمی مورد توجه قرار گرفت و نمونه خون از ورید وداج ۳۸ رأس گاو مبتلا و ۳۸ رأس گاو سالم اخذ و سطوح سرمی کلسیم، فسفر، منیزیم، سدیم، پتاسیم، کلر، گلوکز، تری گلیسرید، کلسترول، آلبومین، گلوبولین و نسبت آلبومین/گلوبولین تعیین شد، تا ارتباط درجه هیپوکلسمی (زیرگروه a؛ کمتر از ۵ میلی گرم بر دسی لیتر، زیرگروه b؛ بین ۵/۱ تا ٦، زیرگروه c؛ بین ٦/۱ تا ۷، زیرگروه d؛ بین ۷/۱ تا ۸ و زیرگروه e؛ بیش از ۸/۱) با سایر فراسنجه ها مشخص گردد. نتایج نشان داد که سطح سرمی کلسیم و فسفر در گاوان زمین گیر به طور معنی داری کمتر و برعکس سطح سرمی منیزیم بیشتر از گروه شاهد بود (P<۰/۰۵). ارتباطی آماری و معنی دار مابین میزان فسفر دریافتی و نسبت k/Ca+Mg با میزان وقوع تب شیر به دست آمد (P<۰/۰۵). در زیر گروه a، میزان کلسیم و گلوکز به طور معنی داری به ترتیب کمتر و بیشتر از سایر زیر گروه ها برآورد گردید (P<۰/۰۵). با توجه به نتایج حاصله از تحقیق حاضر مشخص می شود که نقش هیپوفسفاتمی در به درازا انجامیدن زمین گیری نقشی مؤثر دارد و علاوه بر آن میزان پاسخ به درمان مبتلایان به تب شیر را کاهش می دهد. همچنین حضور ارتباط منفی و معنی داری مابین سطح سرمی کلسیم و پتاسیم و همراهی هیپرگلیسمی با هیپوکلسمی شدید (زیر گروه a) روند عادی درمان تب شیر را متأثر می سازد.**

**واژه‌گان کلیدی**: تب شیر، هیپوکلسمی زایمانی، هیپوفسفاتمی، زایمان، گاو شیری.