



## Calcium metabolism around time of parturition in dairy cows fed on concentrates in Egypt

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### Abstract

The objective of this experiment was to determine the acid-base-balance and calcium metabolism in dairy cows feed on concentrates in Egypt. 40 late pregnant cows (more than 240d), having completed three or more lactations, with an expected calving date within the next three weeks were selected. The animals were randomly allocated to 2 groups which were offered the same diet first group consists of 20 native breed and second group consists of 20 Holstein cows. Mean age and body weight did not differ between the groups. Sample of blood and urine were collected 21 days before the expected day of parturition from each cow weekly, on the day of calving and the next two days Creatinine, urine pH, Fractional excretion and Net acid-base excretion (NABE) were measured in blood and urine.

**Keywords:** Hypocalcaemia, milk fever, calcium homeostasis, calcium mobilization, dairy cow, subclinical acidosis, DCAD.

### Introduction

Milk fever (hypocalcaemia; parturient paresis) is still one of the diseases, of major economic importance to the dairy industry. It is caused by a temporary imbalance between calcium (Ca) supply and demand at the time of parturition. The daily body turnover of Ca changes from < 30 g in nonlactating cows to > 30 g in lactating cows. The resulting low blood Ca levels lead to classic milk fever symptoms within 72 h after calving (Goff et al., 1991; Payne, 2013).

Current evidence suggests that milk fever may occur in cows as a result of excessive dietary cations. High cation diets may cause milk fever in dairy cows as they induce a metabolic alkalosis reducing the ability of the cow to maintain calcium homeostasis at the onset of lactation. Milk Fever (total blood Ca < 1.4 mmol/L) as well as sub-clinical hypocalcaemia (total blood Ca 1.4–2.0 mmol/L) are risk factors for many other diseases connected to lactation including mastitis, ketosis, retained placenta, displaced abomasum and uterine prolapse. Hypocalcaemia is also a risk factor for reproductive disorders and is an indirect risk factor for increased culling (Degaris and Lean, 2009; Goff, et al., 2014; Hunter, 2015). Heifers serum Ca concentration was maintained at a consistently higher level than for multiparous cows (Chan et al., 2006; Carneiro, et al., 2016).

**Calcium homeostasis in the dairy cow**  
Hernandez, and Weaver, (2016) Blood Ca in the adult cow is maintained between 2.1 and 2.5 mmol/L. Typically, the nadir in blood Ca concentration occurs between 12 and 24h after

calving and blood samples obtained around this time can reveal the extent of hypocalcaemia (Yamagishi et al., 1996). Nearly 25 % of heifers will have blood Ca concentrations <2 mmol/L. About 50 % of older cows fall into this category (Goff, 2008). A 500-kg cow needs up to 31 g Ca in order to meet the daily maintenance and demands of the foetus in late gestation (Goff et al., 1991). Cows producing 10 L of colostrums loose about 23 g of Ca in a single milking. This amount is about nine times higher than the entire plasma Ca pool of the cow (Horst et al., 1997).

In order to prevent blood Ca from decreasing at the onset of lactation the cow must replace the Ca losses to the milk. She does this by withdrawing Ca from the bones and by increasing the efficiency of absorption of dietary Ca. The dairy cow is programmed to go into a state of lactational osteoporosis, mobilizing bone Ca to help her achieve normocalcaemia in early lactation. This will typically result in losses of 9–13 % of her skeletal Ca in the first month of lactation (which is reversible later in lactation). Bone Ca mobilization is regulated by parathyroid hormone (PTH) which is produced whenever there is a decline in blood Ca. Renal tubular reabsorption of Ca is also enhanced by PTH. However, the total amount of Ca that can be recovered by reducing urinary Ca excretion is relatively small as only small amounts of calcium are typically lost to urine each day. A second hormone, 1,25-dihydroxyvitamin D<sub>3</sub>, is required to stimulate the intestine to efficiently

absorb dietary Ca (Hernandez, and Weaver, 2016; Yamagishi et al., 1996). This hormone is made from vitamin D by the kidneys (Goff, 2008).

#### **Net acid-base excretion (NABE)**

(Donat, et al., 2016; Rerat, and Schlegel, 2014), For determination of NABE, two variations can be used: the fractional NABE and the simple NABE (Bender et al. 2003; Lachmann, 1981).

The determination of NABE is based on the idea of titration. The titration determines the value or the concentration of the unknown substance, by using substances of known concentration, with a defined pH value of discolouration of the indicator (Donat, et al., 2016; Rerat, and Schlegel, 2014).

In the fractional NABE, HCl can determine the total excreted bases, NaOH the total content of acids e.g., after fixation by formaldehyde also the quantity of titrated  $\text{NH}_4$  (Lachmann, 1981). Based on the received quantities, the content of excreted bases, acids,  $\text{NH}_4$ , the acid base balance and the NABE may be calculated. Using these results the acidosis or alkalosis situation of the animal could be evaluated (Davenport, 1973).

#### **Monitoring the Calcium metabolism around time of parturition**

The monitoring of the Calcium metabolism around time of parturition is necessary because cows might consume less amount of diet due to the physiological changes of late pregnancy and parturition Loor, et al., (2013).

Changes in urine pH as an index of body acid-base status has proved a valuable and inexpensive means of monitoring the success of addition of anions to prepartal rations to prevent milk fever in the field (Gaynor et al., 1989; Davidson et al., 1995; Jardon, 1995). To monitor a sufficient effect of anionic salts, urine samples have to be analyzed for pH & net acid-base excretion (NABE) (Gelfert et al., 2007). Urinary pH was very indicative of changes in the acid-base status of dairy cows with DCAD, (Vagnoni and Oetzel, 1998; Hu and Murphy, 2004). Urine pH generally reflects the acid-base status of an animal. Many investigators measure the urine pH of pre-fresh cows (cows in the final three weeks prior to their due date) to monitor the effectiveness of a ration containing anionic salts.

Urine pH on high cation diets is generally above 8.2. Limiting dietary cations will reduce urine pH only a small amount (down to 7.8). For optimal control of subclinical hypocalcaemia the average pH of the urine of Holstein cows should be between 6.2 and 6.8, which essentially requires addition of anions to the ration. In Jersey cows the average urine pH of the close-up cows has to be reduced to between 5.8 and 6.3 for effective control of hypocalcaemia. If the average urine pH is between 5.0 and 5.5, excessive anions induced an uncompensated metabolic acidosis and the cows will suffer a decline in dry matter intake (Goof, 2008; Goff, 2014).

#### **Materials and methods**

Blood samples were drawn from the vena jugularis into Vacutainer tubes (Vacutainer, silicone coated). A sample of blood was collected from each cow weekly, on the day of calving and the next two days. All blood samples were taken at 07:00 about one hour after the cows finished the morning feed. Samples were stored on ice during transport and centrifuged immediately after arrival at the laboratory. All blood samples were centrifuged for 10 min at 4000 g. The serum was separated into polyethylene tubes serum and stored at  $-20\text{ }^\circ\text{C}$  immediately until the analyses were performed.

Urine Samples, The cows were manually stimulated to urinate by gentle massaging of the perineum, one day each week before parturition, at the day of parturition and daily after parturition for 2 consecutive days, at approximately the same time of blood sampling for the duration of the experiment. When stimulation to urinate failed, urine samples were collected using a sterile Rüschi® catheter after washing the vulva with a septic soap (Betadine, Provect AG, Lyssach, Switzerland). A sample of midstream urine was collected in a 30 ml container. 2 ml of the sample were separated in Eppendorf tubes and frozen for subsequent Ca and Creatinine analysis. The rest was frozen in the container for further analysis of pH and net acid base excretion (NABE). Calcium Chemical urine analyses were performed on centrifuged urine samples by a fully selected chemistry Autoanalyzer Hitachi 911® (ROCHE Diagnostics, Vienna, Austria). The methods were applied according to the manufacturers' recommendations. Quality control material

was analyzed prior to each run to check adequate function of the assays. Creatinine, Urine creatinine was determined by an enzymatic assay with automated predilution and Urine-Calcium by a chromogenic test with o-Kresolphthalein. Urine pH as determined immediately, using a pH meter calibrated, with pH 7.0 and 10.0 buffers (WTW, Weilheim, Germany).

**Calcium**

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**Creatinine**

Urine creatinine was determined by an enzymatic assay with automated predilution and Urine-Calcium by a chromogenic test with o-Kresolphthalein.

**Fractional excretion**

Fractional excretion of Ca was calculated using the following formula:

$$FE\ Ca\ \% = \frac{\text{Urinary Ca} / \text{Serum Ca}^{2+}}{\text{Urinary Creatinine/ Serum Creatinine}} \times 100$$

Creatinine

**Net acid-base excretion (NABE)**

Urinary bases, urine acids and ammonium were measured by titration (table 1) of urine samples according to Kutas (1965). After titration NABE was calculated by using the concentrations of bases, acids and ammonium as follows:

$$NABE\ (mmol/l) = 10 \times (10 \times \text{bases}\ (mmol/l) - (\text{acids}\ (mmol/l) + \text{ammonium}\ (mmol/l))).$$

**Table 2:** Determination of NABE by Kutas (1965):

Fractional NABE	
-	10 ml urine is titrated with 1n HCl to a pH of 3,5
-	30 seconds heating
-	cooling
-	titration with 0,1 n NaOH to a pH of 7,4
-	10 ml 20% Formaldehyd solution added
-	titration with 0,1 n NaOH to a pH of 7,4

Bases (mmol/l) = Volum of HCl × 100
Acids (mmol/l) = Volum of NaOH1 × 10
NH <sub>4</sub> (mmol/l) = Volum of NaOH2 × 10
NABE (mmol/l) = (Volum of HCl × 10 - (Volum of NaOH1 + Volum of NaOH2)) × 10

**Statistical analysis**

ANOVA was used. In this analysis, the Calcium homeostasis was considered the main effects and the cow was a random factor. Dunnett-t-test was carried out as a post-hoc-test to compare the changes of each test day, to day zero. The level of significance was fixed at p= 0, 05 for each single parameter.

**Results**

**Table 1:** Composition of the dry ration in Kg, for pregnant dairy cows and the dietary cation-anion difference (DCAD) in the first group (CG) and second groups

	first group	second group
Alfalfa Silage	8	8
Hay 1st Cut	5	5
Hay	2	2
Concentrates	1,5	1,5
Dry matter intake	10,92 kg/d	10,92 kg/d

Quantities of Elements in %	per kg dry matter (DM)	
Potassium	3,51	3,51
Sodium	0,19	0,19
Chlorid	0,63	0,63
Sulfur	0,22	0,52
DCAD (mEq)	610	613

**Table 2:** Total numbers of cows, incidence of milk fever

Groups	Total numbers	Recumbancy
first group	20	2

Second group	20	4
Total	40	6

**Table 3:** Mean concentration of different parameters in serum and urine of dairy cows in the last three weeks before parturition

Group	Day	Urinary pH	B as e	A c i d	N H <sub>4</sub> <sup>+</sup>	N A B E	S e r u m C a	U r i n a r y C a	F E C a
First group	21	8,	32	77	3,	27	2,	0,	0,
	14	8,	36	96	4,	26	2,	0,	0,
	7	8,	34	91	3,	24	2,	0,	0,
	Ca	8,	28	72	20	19	1,	0,	0,
	1d	8,	33	85	6,	24	1,	0,	0,
Second group	2	8,	32	75	13	23	2,	1,	0,
	21	8,	31	95	4,	21	2,	1,	0,
	14	8,	27	10	4,	16	2,	2,	0,
	7	8,	26	97	11	15	2,	1,	0,
	Ca	7,	17	79	6,	91	1,	0,	0,
1d	8,	27	82	4,	18	1,	0,	0,	
2	8,	33	10	4,	23	1,	0,	0,	

**Table 4:** Results of the Post-hoc-Test showing the statistical significance between the 2 groups.

Group	Group	Urinary pH	N A B E	B a s e	A c i d s	C a s e r u m	U r i n a r y C a	F E C a
1st	2nd	0,031	0,079	0,323	1,000	1,000	0,770	0,856

### Discussion

These results agree with the study of Goff et al. (2004). Urine pH is easily measured and has proven useful in the field to adjust dietary cation-anion difference (DCAD). Also in agreement with the results of this study, Seifi et al. (2004) reported high urine pH in normal

cows (>8.0). A decrease from the normal pH values from 8.0 to 7.4 indicates an increase in dietary acidity.

Shire, and Beede, (2013), The optimal pH in the urine for the prevention of milk fever has not been clearly defined. Jardon (1995) considered that a pH of 6 to 7 was optimal, whereas Horst et al. (1997) proposed 5.5 to 6.2. Horst et al. (1997) also considered that a pH <5.5 should be avoided because it might indicate that the metabolic acidosis is close to being uncompensated. Urinary pH of 6–7 was optimal for Holstein cattle and a pH of 5.5–6.5 was optimal for Jersey cattle to indicate metabolic acidosis. Charbonneau et al. (2006) concluded that a urinary pH of 7.0, regardless of breed, may be more appropriate (Degaris and Lean, 2009).

Urinary pH was very indicative of changes in the acid–base status of dairy cows with DCAD, especially when DCAD was low or negative (Vagnoni and Oetzel, 1998; Hu and Murphy, 2004).

Goff and Horst (1997) proved urinary pH to be an easy and sensitive mean of monitoring the acid base status of cows, shortly before calving. Urine pH has the advantage of being more stable and less expensive than blood gas and pH analysis. Urine pH may also prove more sensitive than blood pH, because blood pH was unable to distinguish between cows, fed the 2.1 and 3.1% K diets.

The pH of urine generally reflects the acid–base state of an animal, monitoring the pH of urine is an inexpensive and sensitive method to monitor the effect of the diet on the pH of blood and assess the risk of milk fever (Goff and Horst., 1998).

The urinary pH is an effective indicator of the extracellular fluid acid–base balance, and multiparous Holstein cows in late gestation may benefit from consuming negative DCAD diet, for blood calcium homeostasis and improvement of the health status (Wu et al., 2008).

### Calcium concentration in serum

No changes in mean values of serum calcium concentration during the gestation period were noticed between the two groups. At the day of parturition calcium levels decreased remarkably in the groups.

The results disagree with the research of Block (1994) and Tucker et al. (1991). In response to

the metabolic acidosis. Significant changes in total calcium concentrations were only observed in studies on pregnant cows (Block, 1984; Oetzel et al., 1988; Moore et al., 2000) (Hunter, 2015; Goff, 2014). The nadir of plasma Ca observed on the day of calving is due to the highly increased demand of blood Ca for colostrum production (Kume et al., 2003). The findings of the present study agree with studies by other authors. They did not find changes in serum calcium concentrations (Block, 1991; Tucker et al., 1992; Leite et al., 2003; Vagnoni and Oetzel, 1998). As the calcium metabolism is strictly controlled by hormones (Hartmann and Bandt, 2000; Martens, 1995), it might depend on the time the check for alterations of calcium concentrations is carried out, whether the findings are statistically provable.

#### Urinary calcium concentration

The mean values of urinary calcium concentration in the 2 groups during the late

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التمثيل الغذائي للكالسيوم حول وقت الولادة في الأبقار تتغذى على المركزات في مصر

ناجي المشد . سامح عرابي . نهى يوسف . محمد رفعت

اجريت تلك الدراسه بهدف تحديد الاتزان بين الاحماض والقويات وتمثيل الكالسيوم في الإبقار الحلابه التي تتغذى علي المركزات في مصر . تم استخدام 40 بقره عشار ثقيل . تم تقسيم الحيوانات الي مجموعتين كل مجموعه مكونه من 20 بقره ( مصريه في المجموعه الاولى - هولشتين في المجموعه الثانيه) تم اخذ عينات دم وبول في اليوم 21 قبل الولاده لقياس مستوى الكرياتنين والاس الهيدروجيني في البول