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# EFFECT OF DIFFERENT CONCENTRATIONS OF ANIONIC SALT ON URINE PH, ACID-BASE-BALANCE AND CALCIUM METABOLISM IN DAIRY COWS

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## SUMMARY

The objective of this experiment was to determine the influence of calcium sulfate as a new tasteless anionic salt on the acid-base-balance and calcium metabolism in dairy cows, consuming feed rations with a high concentration of potassium. Calcium sulfate is added in different concentrations to obtain a variable DCAD. 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 from the herd. The animals were randomly allocated to 4 groups which were offered the same diet and no anionic salts (control group) or defined amounts of anionic salts in the treatment groups (TG). Mean age and body weight did not differ between the groups. Group one consisting of 10 cows used as control, the second group consisting of 10 cows and treated with 800 g/day of the salt, the third group consisting of 10 cows and treated with 1000 g/day of the salt and the forth group also consisting of 10 cows and treated with 1200 g/day of the salt. The DCAD was calculated according to the following formula (Na + K) – (S + Cl). Monthly samples of feed components were taken for analysis and recalculation of DCAD to give the possibility to correct the amount of anionic salt added. Creatinine, urine pH, Fractional excretion and Net acid-base excretion (NABE) were measured in blood and urine.

**Key words**: Hypocalcaemia; milk fever ; calcium homeostasis; calcium binding; dairy cow; subclinical acidosis; DCAD; anionic salt.

## INTRODACTION

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). 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. Adding anions to the diet may offset the effect of cations forages by inducing a mild metabolic acidosis, restoring the ability to maintain calcium homeostasis. 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).

Anionic salts have been defined as salts higher in the fixed ions Cl and S (anions). They increase absorption of Ca through the gastrointestinal tract and increase bone mobilization of Ca because of their acidifying properties (GANT et al., 1998). Since the anionic salts are relatively easy to handle, they are the components of choice for overcoming the alkalinizing potential of prepartum diet. However, anionic salts may be unpalatable and are always accompanied by a cation, which, depending on its rate of absorption, will negative some of the effects of the anions (GOFF and HORST, 1998). Anionic salts are added to the diet of dry cows 2–3 weeks before calving to achieve a mild metabolic acidosis. The main disadvantage of anionic salts is the lack of palatability and the resulting decrease of feed intake (GOFF and HORST, 1998). Anionic salts can be administered to prevent milk fever without danger of significantly reducing the transfer of Selenium from the dam to the calf and without compromising the Selenium status of the cow when the anionic salts are limited to administration for two to three weeks before calving (GANT et al., 1998). Heifers do not benefit from anionic salts because their serum Ca concentration was maintained at a consistently higher level than for multiparous cows (CHAN et al., 2006).

The monitoring of the use of anionic salts is necessary because cows might refuse to consume the diet due to the inherent bad taste of anionic salts (OETZEL and BARMORE, 1993). As DCAD declines, blood pH decreases

and calcium homeostasis improves. Monitoring 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). The addition of anionic salts to a ration induces metabolic acidosis (GOFF and HORST, 1998), resulting in a reduction of the pH in urine (JOYCE et al., 1997; OETZEL, 1991). Monitoring urine pH has proven useful in the field as a means of monitoring the acidification of the blood caused by anion supplementation; however, it is not foolproof. Sulfate salts were able to acidify the urine to the same extent as the chloride salts but did not acidify the blood to the same extent (GOFF et al., 2004). 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). Urine pH of the cows provides a cheap and fairly accurate assessment of blood pH (HUSBAND and VECQUERAY, 2007), and can be a good judgment of the appropriate level of anion supplementation (JARDON, 1995). Urinary pH is a good indicator to monitor implementation of dietary anionic salts and blood CAD can also be a useful measure (CHAN et al., 2006). 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). Monitoring urine pH is a feasible method for determining the animal's response to dietary anions. 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. If the appropriate amounts of anionic salts are consumed in the ration, the urine pH will be 6.5–5.5 (GAYNOR et al., 1989; GOFF and HORST, 1998; JARDON, 1995).

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).

## MATERIALS AND METHODS

Anionic salt, Natural calcium sulfate ($CaSO_4$) with a grain size of 10 µm was used in the present study. The salt is available in Germany by the name, Transifit" (Dr.Pieper GMBH, Wuthenow Germany). DCAD Calculation In the present study the following equation (Na + K) – (Cl + S) was used for calculation of DCAD. This equation may not be the proper equation for the calculation of the DCAD especially when using calcium sulfate in very high concentrations, to lower the value of DCAD of high potassium fed. 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 0700 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 ˚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 Rüsch® catheter after washing the vulva with a septical soap (Betadine, Provet 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**
was determined immediately, using a pH meter calibrated, with pH 7.0 and 10.0 buffers (WTW, Weilheim, Germany).

**Statistical analysis**
Studies with rumen fistulated cows have shown that 10 cows per group are sufficient for statistical analysis (FRÖMER, 2004; LÖPTIEN, 2004). The changes in the parameter of acid-base-balance and major element metabolism, induced by the anionic salts, are sufficient to minimize the risk of an error of type 2. To compare the reaction of acid-base-balance and calcium metabolism a repeated ANOVA was used. In this analysis, the amounts of the anionic salt added to the feed ration were 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

**Figure 1:** changes in urinary pH in the 4 groups during feeding anionic salts to pregnant dairy cows (n=40)

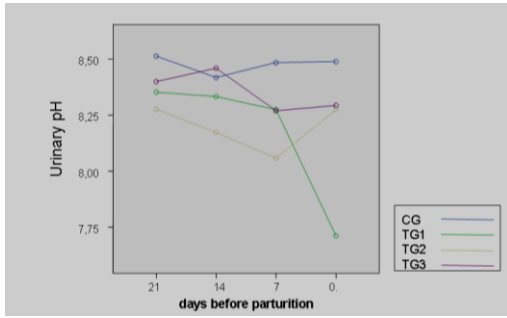

**Figure 2:** changes in serum calcium concentration in the 4 groups during feeding anionic salts to pregnant dairy cows (n=40)

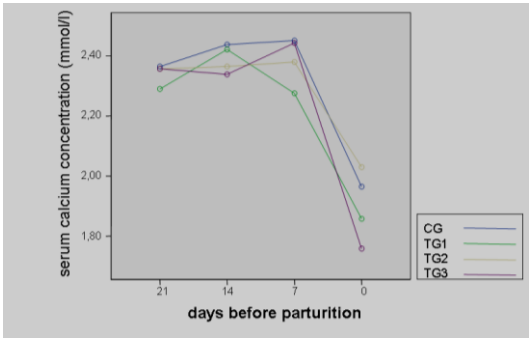


**Figure 3:** changes in urinary calcium concentration in the 4 groups during feeding anionic salts to pregnant dairy cows (n=40)

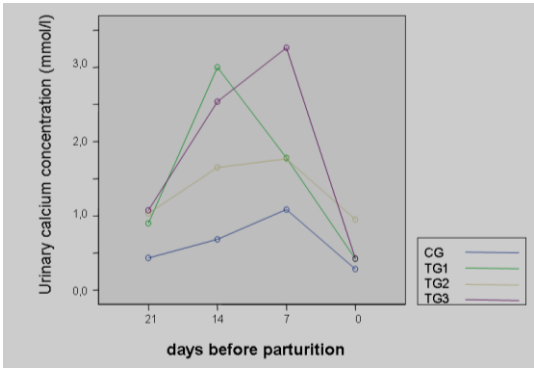


**Table 1:** Results of the Post-hoc-Test showing the statistical significance between the four treatment groups.

| Group | | Urinary pH | NABE | Base | Acids | Ca serum | Urinary Ca | $FE_{Ca}$ |
|---|---|---|---|---|---|---|---|---|
| CG | TG1 | **0,031** | 0,079 | 0,323 | 1,000 | 1,000 | 0,770 | 0,856 |
| | TG2 | 1,000 | **0,007** | **0,011** | 0,790 | 1,000 | 1,000 | 0,255 |
| | TG3 | 1,000 | 0,056 | 0,132 | 0,677 | 1,000 | 0,167 | **0,045** |
| TG1 | TG2 | 0,401 | 1,000 | 1,000 | 0,285 | 1,000 | 1,000 | 1,000 |
| | TG3 | 0,226 | 1,000 | 1,000 | 1,000 | 1,000 | 1,000 | 1,000 |
| TG2 | TG3 | 1,000 | 1,000 | 1,000 | **0,019** | 1,000 | 1,000 | 1,000 |

**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). However, it does not always accurately assess the degree of acidosis induced by chloride or sulfate addition to the diet. For routine monitoring of the dry cow, the ease of measuring urine pH, more than makes up for its inaccuracy. 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. 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).

The kidney can efficiently eliminate excess anions from the blood, thus addition of anionic salts induces a sharp reduction in urinary pH. This was associated with a mild metabolic acidosis (JOYCE et al., 1997; PEHRSON et al., 1999). Similar results have been observed in previous studies for dairy cows (VAGNONI and OETZEL, 1998; MOORE et al., 2000; LIESEGANG et al., 2007) and buffalos (SHAHZAD et al., 2008). On the other hand, DCAD has been proven to be associated with fluid acid–base balance. SPANGHERO (2004) found a strong relationship between DCAD and urinary pH in support of CHARBONNEAU et al. (2006). The established weak association between DCAD and blood pH may be due to the difference in buffering capability between the blood and urine. Urinary pH, due to its high sensitivity and ease of assessment on the farm, may be a simple and efficient monitor of the acid–base balance in extracellular fluids (WU et al., 2008).

In conclusion, the use of anionic salts results in a decrease of urine pH, the unexpected value of urine pH in this study may be due to the use of high

amounts of anionic salt to lower the DCAD of the ration, while the normal hay used contain very high amounts of K. The high amount of anionic salts decrease food intake, hence the cows received less amount of anionic salt.
A possible explanation was an incorrect mixing of the anionic salt containing premix, an inconsistent intake of feed, because of the cows being able to separate the premix from the other feed components.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).

Blood pH, theoretically less able to discern the effects of diet on metabolic alkalosis and acidosis, is more commonly measured. Urine pH is easily measured and has proven useful in the field to adjust the dietary cation-anion difference (DCAD). However, it does not always accurately assess the degree of acidosis induced by chloride or sulfate addition to the diet. For routine monitoring of the dry cow, the ease with which urine pH can be measured more than makes up for its inaccuracy (GOFF et al., 2004).
Commonly, urine pH is considered an adequate parameter for monitoring the anionic salt intake of cows (OETZEL, 2002).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).

Feed, containing anionic salts and still revealing a positive DCAD, induces a metabolic acidosis and activates calcium mobilization, as an effect of a compensation mechanism (BENDER et al., 2003). The results of such field studies have to deal with fluctuations in the composition of feed and feed

intake of cows (GOFF et al.,2004) and reliable results are dependent of optimal feeding management (HUSBAND et al., 2002). Unnoticeable acidogenic effects would result in similar effects regarding urine composition (OWENS et al., 1998). Clinical acidosis occurs when the dosage of anionic salts is too high, or the resulting DCAD is too low (GELFERT et al., 2006). A certain level of acidosis is necessary to activate the calcium metabolism.

After feeding the anionic salt, no changes in mean values of serum calcium concentration during the gestation period were noticed between the different TG. At the day of parturition calcium levels decreased remarkably in all groups.The results disagree with the research of BLOCK (1994) and TUCKER et al. (1991). In response to the metabolic acidosis, the authors found slight and significant increases of serum concentrations. Significant changes in total calcium concentrations were only observed in studies on pregnant cows (BLOCK, 1984; OETZEL et al., 1988; MOORE et al., 2000). TAKAGI and BLOCK (1991) obtained similar results of calcium concentrations by simulating a greater calcium loss by an EDTA infusion.
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 declined degree of plasma Ca was lower in TG 2, when the cows consumed 1000 g of calcium sulfate. Similar findings were reported by CHARBONNEAU et al. (2006) and LEAN et al. (2006).

The findings of the present study agree with studies by other authors. They did not find changes in serum calcium concentrations (TAKAGI and 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. GELFERT et al. (2006) monitored the impact of anionic salts in a similar study. According to their results, DCAD must be lower than 160 mEq/kg DM to affect ABB (acid-base balance) and calcium mobilization sufficiently (GELFERT et al., 2007).

After feeding the anionic salt a marked increase in mean values of urinary calcium concentration in the three TG during the late gestation period was visible. However, there were no significant differences between the treatment groups and the CG. The results of the present study disagree with the findings of ROCHE et al. (2002), who reduced DCAD from +400 to +350 mEq/kg DM in linearly increased ($P < 0.05$) Ca/Creat. This may be an indicator for increased intestinal absorption, bone resorption or a reduced renal reabsorption, despite unaffected plasma Ca concentration. Such an effect was unexpected because such a small decrease in DCAD did not affect the systemic pH (as measured by urine pH) and would not be expected to influence Ca homeostasis (ROCHE, 1999; UNDERWOOD and SUTTLE, 1999; GOFF, 2000). In the same experiment ROCHE et al. (2002) found larger changes in DCAD without significant changes of Ca/Creat, even though there was a linear decline of urine pH.

In other studies calcium concentrations in urine increased and differed significantly, beyond the feeding of anionic salts. The increase of calcium concentrations in urine is described by FREEDEN et al. (1988), VAGNONI and OETZEL (1998), BENDER et al. (2003) and ROCHE et al. (2003). In some studies the increase of calcium excretion is found to be a result of the oversupply of feeding lime, to fulfill the need of calcium when anionic salts are fed (BYERS, 1994; BEENING, 1998). The oversupply of calcium increases the rate of passive absorption in the intestine, resulting in an oversupply of calcium in the blood, which is regulated by the kidney (WANG and BEEDE, 1992; HARTMANN and BANDT, 2000). SHAHZAD et al. (2008) increased urinary Ca excretion in buffaloes by feeding a diet with -11 DCAD concentration. This may be due to the slight metabolic acidosis that not only increased intestinal Ca absorption (SCHONEWILLE et al., 1994; ROCHE et al., 2003) but also Ca resorption from the bones due to an increased synthesis of $1,25(OH)_2D_3$ (GOFF et al., 1991). The acidosis maintains a high Ca flux through the exchangeable pool without affecting the pool size (FREDEEN et al., 1988). Reduced urinary Ca excretion with increased concentrations of DCAD may be due to the gradual vanishing effect of metabolic acidosis.

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