EFFECT OF PRE-PARTUM DIETARY CATION-ANION DIFFERENCE ON THE PERFORMANCE OF TRANSITION SAHIWAL CATTLE

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ABSTRACT

Dietary cation anion difference (DCAD) is an important aspect of dairy nutrition, especially in the transition period. Sahiwal cattle is the highest milk producing breed among Zebu cattle. A study was planned on transition Sahiwal cattle to determine the effects of feeding varying levels of negative DCAD. For this purpose, twenty pregnant cows (at the 250th day of gestation) were selected and randomly divided into 5 groups comprising four animals each. Five iso-caloric (2100 Kcal) and iso-nitrogenous (12%) diets were formulated and each diet was allotted to each group. The animals who received positive DCAD diet (+134.32 mEq/Kg DM) served as control. Diets were supplemented with NutriCAB® to attain 0, -15, -30 and -45 mEq/kg DM DCAD levels. Experimental diets were fed at ad-libitum up to parturition and data regarding feed intake were recorded daily. Post-partum incidence of milk fever, dystocia, retention of placenta (RP), mastitis as well as milk production, milk fat percentage and serum Ca levels were recorded. Urine and blood pH were determined weekly during the last month of pregnancy. Results showed that prepartum feed intake and blood pH were not affected (P>0.05), while urine pH was significantly reduced (P<0.05) by lowering DCAD levels. Post parturient blood calcium level linearly increased (P<0.05) with decreasing DCAD. Pre-partum negative DCAD feeding had no significant effect (P>0.05) on post-parturient milk production and fat percentage. However, subclinical milk fever, RP and clinical mastitis decreased with decreasing DCAD feeding. It was concluded that negative DCAD feeding raised serum calcium level and reduced the incidence of post-parturient problems in Sahiwal cattle.

Key words: Dairy cattle, transition, metabolic diseases, reproductive disorders, incidence.

Abbreviations used: DCAD = (dietary cation anion difference), Ca = (calcium), RP = (retention of placenta).

INTRODUCTION

Efficient feeding of dairy cows during transition period is a key for successful dairy enterprise. Transition period of the dairy cow is important for milk production and dairy cow health (Keady et al., 2001). This period covers three weeks before and three weeks after parturition. Energy requirement, endocrinological and metabolic changes occur during this period associated with dietary cation anion difference (DCAD) (Piccione et al., 2012). Maintenance, growth, gestation and lactation determine the demand of dietary minerals (Nielsen and Volden et al., 2011). Diets having negative DCAD create acidic conditions in body results in improved parathyroid hormone functions. Minerals play an important role in body physiology and acid-base balance (Afzaal et al., 2004). The pH of biological fluids is directly linked with biochemical reactions essential for life support functions. Positively charged minerals impact alkalogenically; whereas, the negatively charged minerals have acidogenic effect on body fluids (Block, 1994). High pH of blood reduces the receptivity of tissues (bones and kidney) for parathyroid hormones (PTH). This condition results in lower calcium mobilization from bones and poor conversion of Vitamin D into 1, 25-dihydroxyvitamin D by kidneys, causing negative effect on Ca absorption from gastrointestinal tract (Goff, 2008).

Inability of dairy animal to maintain normal Ca results in milk fever (De Garis and Lean, 2008). Sub-optimum Ca homeostasis increases the risk of periparturient problems such as dystocia, retained placenta (RP), metritis, mastitis (Mulligan et al., 2006). Various prophylactic measures are in vogue to prevent milk fever. These techniques includes oral drenching or feeding of lower Ca diet (<20g/day), administration of vitamin D and feeding of lower DCAD diet (Thilsing et al., 2002). Recent research advocates the benefits of DCAD feeding to attain the desired blood ionic Ca level and to reduce milk fever and parturient problems (Shire and Beede, 2013). Usually, calcium chloride (CaCl₂) is given as anionic salt. It raises the level of chloride ions (Cl⁻) without rising sodium or potassium ions (Na⁺, K⁺) causing acidifying effect (National Research Council, 2001). Irritation, bitterness and poor palatability are the problems associated with CaCl₂ supplementation (Lawless et al., 2003; Scott and Vijk, 2000; Scott and Vijk, 2002; Tucker et al., 1991). To overcome this problem anionic salts are always mixed with feed to avoid reduction in feed intake (Goff, 2008). We used a commercially available negative DCAD diet “NutriCAB®”. This anionic product also avoids reduction in feed intake
encapsulated in pan coating to ensure better palatability and intestinal release without affecting feed intake.

Sahiwal cattle is the most important cattle milk breed of Pakistan including tropical regions of world; having highest milk production among Zebu cattle with docile temperament, heat tolerant, tick, drought, parasite and bloat resistance and ease of calving characteristics. Keeping in view the above mentioned scenario, we aimed first study to investigate the effects of feeding different levels of negative DCAD diet “NutriCAB®” on productive performance, Ca status and postpartum problems in Sahiwal cattle.

MATERIALS AND METHODS

Experimental animals and feeding: The experiment was conducted at Livestock Experiment Station, Khizerabad, Sargodha, Pakistan for 90 days (30 days before and 60 days after parturition) during August to October, 2015. Twenty pregnant Sahiwal cows (3rd parity) at their last months of pregnancy were selected and randomly divided into five groups comprising four animals each under randomized complete block design (RCBD). The diets were formulated having 2100 Kcal energy and 12% protein according to NRC recommendations (Table 1; National Research Council, 2001). Each diet was randomly allotted except control who received positive DCAD. Diets were supplemented with NutriCAB® (Table 2) to attain 0,-15,-30,-45 mEq/Kg DM DCAD levels (Table 3). The experimental animals were kept on concrete floor in an isolated shed for 15 days of adaptation period. After adaptation cows were tied and experimental diets were provided at *ad-libitum* up to 4 weeks before parturition. After parturition the animals were fed according to their production performance. Concentrate was fed at the rate of 2Kg/animal once in a day and chopped (2cm) fresh Rhode grass was fed at *ad-libitum* basis in individual mangers till parturition. Fresh drinking water was available around the clock throughout experiment. However, just after calving experimental animals were fed normal routine diet with positive DCAD (121.3 mEq/Kg).

Sampling: All experimental animals were provided green fodder early in morning and orts were collected in the next morning to calculate feed intake. Feed intake of each animal was calculated as: (Feed intake (Kg) = Total amount of feed offered – feed refused) (Norrish and Hutton, 1977). Daily feed intake was noted from last month of parturition till calving. Post parturient milk production was recorded up to 60 days. Animal were milked twice a day (4:00 am and 3:00 pm) by manual method. Milk production of animals was recorded on daily basis. Milk fat percentage was recorded weekly during first month of lactation. Approximately 100 ml fresh milk sample (50 ml of each time) was taken in a beaker for milk fat determination. Incidence of milk fever was observed during 12 hours before and 7 days after calving using clinical signs and monitoring serum Ca level. The cows who showed clinical signs or <7.5 mg/dl serum Ca level were considered a case of milk fever (Massey et al., 1993; Oetzel et al., 1998). Blood samples were collected weekly at 28, 21, 14 and 7 day before calving. Urine and blood pH were determined weekly during the last month of pregnancy. Five milliliter of blood was collected in gel containing test tube by jugular vein puncture with 16 gauge intravenous needle and serum was separated for pH determination. Blood samples were collected 6 to 12 hours after feeding the experimental diets (Tucker et al., 1991). Midstream urine sample was collected in 100 ml container weekly (day 28, 21, 14, 7) by spontaneous or manually stimulated urination (Van Dijk and Lourens, 2001). It was performed 3-4 hours after feeding experimental diet. For determination of serum Ca, blood samples were taken within 24 hours after calving and allowed to clot at room temperature. Serum was separated by centrifugation and kept at 4°C in capped polypropylene tubes (Miles et al., 2001). The experimental cows were monitored for post-partum diseases and disorders including milk fever, dystocia, RP and clinical mastitis.

Laboratory analysis: Gerber method was used to evaluate fat percentage at Nestle Collection Centre, Khizerabad, District Sargodha, Pakistan. Approximately 100 ml milk sample (50 ml per time) was taken in a beaker. Ten milliliter of H2SO4, 10.94 ml milk, 1ml alcohol was added and shaken after insertion of stopper in Butyrometer. Then, it was placed in Gerber centrifuge machine for 5 minutes at 1500 rpm. The fat contents moved on top and milk fat percentage reading was recorded (James, 1995).

For pH determination, a drop of serum/urine sample was taken with help of dropper and placed on pH strip. After one minute color of strip was matched with standard pH value (Spanghero, 2004). Serum Ca was evaluated with semi-automated chemistry analyser (Microlab 300; Massanyi et al., 2007) at University Medical Complex and Research Centre, University of Sargodha, Sargodha, Pakistan.

Extrapolation of economic losses and benefit cost ratio: Losses due to incidence of milk fever, clinical mastitis, dystocia and retention of placenta (RP) were assessed on the basis of following parameters and equations as well as consultation with local veterinarians (Dematawewa and Berger, 1997; Thirunavukkarasu et al., 2010; Ashraq et al., 2015).

Parameters: Loss of milk production
Treatment expenses
Veterinarian charges
Value of discard milk
Loss in animal sale value
Cost of NutriCAB® diet

Equations: Total losses due to milk fever = Value of milk loss + Veterinarian charges + Treatment cost + Loss in animal sale value
Total losses due to retention of placenta = Value of milk loss + Veterinarian charges Treatment cost + Loss in animal sale value
Total losses due to dystocia = Value of milk loss + Veterinarian charges Treatment cost + Loss in animal sale value
Total losses due to clinical mastitis = Value of milk loss + Veterinarian charges Treatment cost + Value of discarded milk + Loss in animal sale value

Benefits from control of a particular disease

The benefit cost ratio = ______________________
Prevention cost of the disease

Statistical analysis: The results were statistically analyzed under RCBD by using general linear model and means of different treatments were separated by Tukey’s test (Steel et al., 1997). In addition to compare means polynomial regression analysis was performed to evaluate the linear or quadratic effects of treatments. The cost benefit ratio was analyzed by simple addition and subtraction (Thirunavukkarasu et al., 2010).

RESULTS

Average feed intake was not affected by decreasing DCAD levels (P>0.05) during last month of pregnancy in Sahiwal cattle (Table 4). During transition period blood Ca had increased linearly (P<0.05) with decreasing DCAD levels in Sahiwal cattle. Highest blood Ca was recorded at -45 DCAD and lowest at positive DCAD (Table 4). Postpartum, total and average milk production had no significant change (P>0.05) by decreasing DCAD diet during early transition phase (Table 4). Milk fat percentage of Sahiwal cattle did not show any change by decreasing the levels of DCAD in diet (P>0.05; Table 4).

Results of present study showed that weekly and average blood pH was not influenced (P>0.05) by negative DCAD feeding in Sahiwal cows (Table 4). Urine pH was not affected (P>0.05) by decreasing DCAD level during 1st and 2nd week of trial. While in 3rd and 4th week a linear decrease (P<0.05) was observed by decreasing DCAD level. However, lowest pH was recorded at -45 DCAD and highest at positive DCAD during 3rd and 4th week of trial (Table 4).

The negative DCAD diet considerably reduced the incidence of postparturient problems in experimental animals viz. subclinical milk fever, RP and clinical mastitis. Whereas; no case of clinical milk fever was observed (Table 5).

Economic losses due to incidence of subclinical milk fever (A) in Sahiwal cattle at Khizerabad, District Sargodha during July to October, 2015:
Subclinical milk fever in control group = 25%
Subclinical milk fever in DCAD treated groups = 6.25%
Reduction in incidence = 25 - 6.25 = 18.75%
Number of animals prevented = 03
Per animal economic losses of subclinical milk fever (A) = 114.3 $
Benefit of subclinical milk fever (A) prevention = 114.3 $
Total benefit of subclinical milk fever (A) = 3×114.3=342.9 $  
Per animal cost of feed = 15.75 $

Economic losses due to dystocia in Sahiwal cattle at Khizerabad, District Sargodha during July to October, 2015:

Cases of dystocia in positive DCAD group = 0%
Cases of dystocia in negative DCAD group = 0%
The DCAD diet had no effect on dystocia.

Economic losses due to incidence of clinical mastitis (C) in Sahiwal cattle at Khizerabad, District Sargodha during July to October, 2015:
Incidence of clinical mastitis in control group = 25%
Incidence of clinical mastitis in DCAD treated group = 0%
Reduction in incidence of clinical mastitis = 25 - 0 = 25
Number of animals prevented = 04
Per animal economic losses of retention of placenta (B) = 07.9 $
Benefit of retention of placenta (B) prevention = 07.9 $
Total benefit of retention of placenta (B) = 4×7.9=31.6 $
Per animal cost of feed = 15.75 $

Over all benefit of using NutriCAB® supplementation:
Total cost of feed supplement (E) = 252$ (@ 15.75 per animal)
Profit due to increase of milk production using NutriCAB® supplementation ($F$) = $104.8$.
Total profit $G = D + F = 580.9 + 104.8 = 685.7$.
Net profit $(G-E) = 685.7 - 252 = 433.7$ ($for 16$ experimental animals except control).

**Benefit cost ratio:**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Benefit ($)</th>
<th>Cost ($)</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk fever</td>
<td>114.3</td>
<td>15.75</td>
<td>7.3</td>
</tr>
<tr>
<td>Retention of placenta</td>
<td>7.9</td>
<td>15.75</td>
<td>0.5</td>
</tr>
<tr>
<td>Clinical mastitis</td>
<td>51.6</td>
<td>15.75</td>
<td>3.3</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Average feed intake was not affected by decreasing DCAD levels $(P>0.05)$ during last month of pregnancy in Sahiwal cattle (Table 4). Similar findings are reported by Heron et al. (2010) and Weich et al. (2013). Modified palatability of commercial products or higher herd dry matter intake or lesser inclusion level of anionic salt would be the outcome for unaffected feed intake (Hutjens, 1991; Oetzel et al., 1991; DeGroot et al., 2010). On the contrary, some researchers depicted that feed intake decreased by lowering DCAD diet (Chan et al., 2006; Luebbe et al., 2011). Reduction in feed intake might be due to systemic acidosis or physiological and endocrinological changes during transition phase (Holtenius et al., 2003; Plaizier et al., 2008).

During transition phase, blood Ca had increased linearly $(P<0.05)$ with decreasing DCAD levels in Sahiwal cattle. Highest blood Ca was recorded at -45 DCAD and lowest at positive DCAD (Table 4). These findings are supported by various researchers who reported that feeding of lower DCAD diet before calving have substantial effect on blood Ca status (Grunberg et al., 2011; Oba et al., 2011; Reece 2009). Results of current study are contradictory to the outcomes, who stated that the blood Ca status remained unaffected by varying DCAD levels during transition phase (Goff and Horst, 2004; Rezac et al., 2014). The Ca and K contents in feed including ecological and environmental stress could be involved for impasse Ca level (Nieves et al., 2009).

Postpartum average milk production had no significant change $(P>0.05)$ by decreasing DCAD diet during early transition phase (Table 4). This might be due to generation of slight acidic environment in rumen by lower DCAD diet (Atkinson, 2014) or genetic makeup (Koivula et al., 2005). Outcomes of current study are similar with Martinez et al. (2012); Weich et al. (2013) and Silva (2015) who demonstrated that prepartum negative DCAD feeding had no effect on postpartum average milk production. Contradictory results were reported by Ganjkhanlou et al. (2010) and Jawor et al. (2012) who reported that post partum milk production was increased by prepartum negative DCAD diet.

Increased milk production might be associated with nutrient composition of postpartum diet and level of production (Rekhis et al., 2001; Suttle and McLauchlan, 2010).

Milk fat percentage of Sahiwal cattle did not show any change by decreasing the levels of DCAD in diet $(P>0.05)$; Table 4). Result of this finding are in agreement with finding of Chamberlin et al. (2013) who mentioned that fat percentage was not affected by different DCAD feeding during early transition period. Conversely, positive DCAD of diet had direct effect on fat percentage (Hu and Kung-Jr, 2009). The improvement in fat percentage after calving might be related to increase in DMI by positive DCAD diet (Hu and Murphy, 2004); this increased the rumen pH and stimulate fermentation process to synthesize volatile fatty acids (Kolver and DeVeth, 2002); consequently de novo synthesis of fatty acid takes place in mammary cells (Roche et al., 2005).

Results of present study showed that weekly and average blood pH was not influenced $(P>0.05)$ by negative DCAD feeding in Sahiwal cows (Table 4, 6). Similar findings were reported by Grunberg et al.(2011) and Ganjkhanlou et al. (2010) while contradictory results were reported by Goff (2008); they described that negative DCAD diet had direct impact on blood pH. The high concentration of anionic salts in diet supported the strong ion difference theory; this help to determine the mechanism of acid-base disorders (Gelfert et al., 2006; Neligan and Deutschman, 2014). Change in Na+, K+ and Cl- ions (strong ions) of extracellular fluid causes alteration in acid based balance (Stewart, 1983).

Urine pH was not significantly affected $(P>0.05)$ by decreasing DCAD level during 1st and 2nd week of trial. While in 3rd and 4th week a linear decrease $(P<0.05)$ was observed by decreasing DCAD level (Table 7). However, lowest pH was recorded at -45 DCAD and highest at positive DCAD during 3rd and 4th week of trial (Table 4). These results are in accord with the finding of Grunberg et al. (2011) and Wu (2011) who stated that diet with lower DCAD level had significant effect on urine pH. The possible reason of reduction in urine pH was the acidifying effect of negative DCAD diet (Pennet et al., 2008; Rerat et al., 2009). Monovalent ions possess high bioavailability that strictly impact blood acid base status (Goff et al., 2004). However, Lean and DeGaris (2010) stated that varying DCAD levels had no impacts on urine pH. Improper consumption of anionic salt could be the possible reason for this result (Block, 2011).

The negative DCAD diet considerably reduced the risk of postparturient problems in experimental animals viz. subclinical milk fever, RP and clinical mastitis. Whereas; no case of clinical milk fever was observed (Table 5); as supported by various researchers (Crnkic et al., 2010; Ghattas, 2014; Klos et al., 2015; Sakha et al., 2014). However, results of present study are
inconsistent with the finding of Melendez et al. (2004), Hu et al. (2007), Gulay et al. (2005) who reported that negative DCAD diet did not reduce the incidence of post parturient problems. Various factors including age, breed, milk production, hormonal changes and type of forages (feeding of forages containing high K and Ca) during early transition (Rehage and Kaske, 2004; Taylor et al., 2008). Demineralization process of bones in dairy cows decrease with increasing age (Sjaastad et al., 2010). Whereas; Goff (2008), Mulligan and Doherty (2008) and Oetzel (2011) described that hypocalcaemia is directly associated with dystocia, RP and mastitis because of the significant role of Ca in muscle functioning and immune system (Shire and Beede. 2013; Santos et al., 2011).

Table 1. Ingredient composition of experimental ration

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percentage</th>
<th>CP</th>
<th>ME (Mcal/Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotton seed Cake</td>
<td>12</td>
<td>2.58</td>
<td>0.2568</td>
</tr>
<tr>
<td>Linseed Cake</td>
<td>4</td>
<td>1.2888</td>
<td>0.1152</td>
</tr>
<tr>
<td>Sorghum</td>
<td>20</td>
<td>3.15</td>
<td>0.568</td>
</tr>
<tr>
<td>Rhode grass</td>
<td>27</td>
<td>2.43</td>
<td>0.5481</td>
</tr>
<tr>
<td>Wheat Bran</td>
<td>7</td>
<td>0.959</td>
<td>0.1645</td>
</tr>
<tr>
<td>Wheat Straw</td>
<td>27</td>
<td>0.648</td>
<td>0.3888</td>
</tr>
<tr>
<td>Mustard Cake</td>
<td>3</td>
<td>0.969</td>
<td>0.912</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>12.0248</td>
<td>2.1326</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Items (%)</th>
<th>DCAD³</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Positive</td>
<td>1</td>
</tr>
<tr>
<td>Retention of placenta</td>
<td>1</td>
</tr>
<tr>
<td>Dystocia</td>
<td>0</td>
</tr>
<tr>
<td>Clinical mastitis</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 2. Analytical constituents of NutriCAB.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Constituents</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Calcium</td>
<td>28</td>
</tr>
<tr>
<td>2</td>
<td>Phosphorus</td>
<td>0.1</td>
</tr>
<tr>
<td>3</td>
<td>Sodium</td>
<td>0.2</td>
</tr>
<tr>
<td>4</td>
<td>Potassium</td>
<td>0.1</td>
</tr>
<tr>
<td>5</td>
<td>Chloride</td>
<td>48</td>
</tr>
<tr>
<td>6</td>
<td>Sulphur</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Table 3. Allotment of treatments to the experimental groups.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>NutriCABg/day per cow</th>
<th>Levelsmeq/Kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>156</td>
<td>-45</td>
</tr>
<tr>
<td>Group B</td>
<td>143</td>
<td>-30</td>
</tr>
<tr>
<td>Group C</td>
<td>129</td>
<td>-15</td>
</tr>
<tr>
<td>Group D</td>
<td>117</td>
<td>0</td>
</tr>
<tr>
<td>Group E</td>
<td>0</td>
<td>+134.32</td>
</tr>
</tbody>
</table>

Table 4. Dietary means for feed intake, blood calcium, milk production, blood and urine pH in Sahiwal cattle.

<table>
<thead>
<tr>
<th>Items</th>
<th>DCAD³</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter intake (Kg)</td>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood Ca mg/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Items</th>
<th>DCAD³</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter intake (Kg)</td>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood Ca mg/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total milk (L in 60 days)  287.9  259.7  290.5  318.9  311.9  27.435  0.57  0.98
Milk production (L/day)  4.8  4.3  4.8  5.3  5.1  0.4573  0.62  0.90
Milk fat (%age)  4.0  4.0  4.0  4.1  4.1  0.353  0.46  0.87
Average blood pH  7.93  7.93  7.93  7.87  7.68  0.1271  *0.041  0.12
Average urine pH  7.93  7.93  7.93  7.78  7.68  0.1271  *0.041  0.12

DCAD\textsuperscript{1} (Dietary Cation Anion Difference) +ive, 0, -15, -30 and -45 meq/Kg of dry matter indicate inclusion of NutriCAB\textsuperscript{®} at the rate of 0, 117, 129, 143 and 156g/day per animal, respectively.

\textit{abc} Within a row, means sharing different superscripts differ significantly (P<0.05).

SE = standard error

Table 5. Effect of dietary cation anion difference on incidence post parturient problems of transition Sahiwal dairy cattle.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Percentage</th>
<th>CP%</th>
<th>ME (Mcal/Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotton seed cake</td>
<td>15</td>
<td>5.85</td>
<td>0.38</td>
</tr>
<tr>
<td>Canola meal</td>
<td>9</td>
<td>3.4</td>
<td>0.25</td>
</tr>
<tr>
<td>Maize grain</td>
<td>43</td>
<td>3.87</td>
<td>1.21</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>16</td>
<td>2.43</td>
<td>0.36</td>
</tr>
<tr>
<td>Molasses</td>
<td>12</td>
<td>0.53</td>
<td>0.23</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Salt</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sodium Bicarbonate</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DCP</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>16.08</td>
<td>2.42</td>
</tr>
</tbody>
</table>

DCAD\textsuperscript{1} (Dietary Cation Anion Difference) +ive, 0, -15, -30 and -45 meq/Kg of dry matter indicate inclusion of NutriCAB\textsuperscript{®} at the rate of 0, 117, 129, 143 and 156g/day per animal, respectively.

Table 6. Effect of dietary cation anion difference on blood pH of transition Sahiwal dairy cattle.

<table>
<thead>
<tr>
<th>Blood pH</th>
<th>Positive</th>
<th>DCAD\textsuperscript{1}</th>
<th>SE</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>-15</td>
<td>-30</td>
<td>-45</td>
</tr>
<tr>
<td>Week one</td>
<td>8.0</td>
<td>8.0</td>
<td>8.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Week two</td>
<td>8.0</td>
<td>8.0</td>
<td>8.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Week three</td>
<td>8.0\textsuperscript{a}</td>
<td>8.0\textsuperscript{ab}</td>
<td>8.0\textsuperscript{ab}</td>
<td>7.5</td>
</tr>
<tr>
<td>Week four</td>
<td>7.75\textsuperscript{a}</td>
<td>7.75\textsuperscript{ab}</td>
<td>7.75\textsuperscript{ab}</td>
<td>7.75\textsuperscript{ab}</td>
</tr>
<tr>
<td>Average pH</td>
<td>7.93</td>
<td>7.93</td>
<td>7.93</td>
<td>7.87</td>
</tr>
</tbody>
</table>

DCAD\textsuperscript{1} (Dietary Cation Anion Difference) +ive, 0, -15, -30 and -45 meq/Kg of dry matter indicate inclusion of NutriCAB\textsuperscript{®} at the rate of 0, 117, 129, 143 and 156g/day per animal, respectively.

NS = non-significant (P >0.05). * = Significant (P <0.05).

Table 7. Effect of dietary cation anion difference on urine pH of transition Sahiwal dairy cattle.

<table>
<thead>
<tr>
<th>Urine pH</th>
<th>Positive</th>
<th>DCAD\textsuperscript{1}</th>
<th>SE</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>-15</td>
<td>-30</td>
<td>-45</td>
</tr>
<tr>
<td>Week one</td>
<td>8.0</td>
<td>8.0</td>
<td>8.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Week two</td>
<td>8.0</td>
<td>8.0</td>
<td>8.0</td>
<td>7.7</td>
</tr>
<tr>
<td>Week three</td>
<td>8.0\textsuperscript{a}</td>
<td>7.7\textsuperscript{ab}</td>
<td>7.25\textsuperscript{ab}</td>
<td>7.0\textsuperscript{b}</td>
</tr>
<tr>
<td>Week four</td>
<td>7.75\textsuperscript{a}</td>
<td>7.3\textsuperscript{ab}</td>
<td>6.8\textsuperscript{ab}</td>
<td>6.3\textsuperscript{b}</td>
</tr>
<tr>
<td>Average pH</td>
<td>8.06\textsuperscript{a}</td>
<td>7.68\textsuperscript{ab}</td>
<td>7.25\textsuperscript{b}</td>
<td>6.8\textsuperscript{ab}</td>
</tr>
</tbody>
</table>

DCAD\textsuperscript{1} (Dietary Cation Anion Difference) +ive, 0, -15, -30 and -45 meq/Kg of dry matter indicate inclusion of NutriCAB\textsuperscript{®} at the rate of 0, 117, 129, 143 and 156g/day per animal, respectively.

\textit{abc} Within a row, means sharing different superscripts differ significantly (P<0.05). NS = non-significant (P >0.05). * = Significant (P <0.05).

**Conclusion:** Blood ionic Ca had improved by providing negative DCAD supplementation. This helped to reduce the incidence of postparturient problems (subclinical milk fever, RP, and mastitis) in moderate milk producing cattle breed i.e. Sahiwal cattle. The supplementation of anionic...
salt would be economical for dairy animals with high incidence of clinical milk fever.

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