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Evaluation of pre-partum Dietary Cation Anion Difference (DCAD) supplementation on calcium homeostasis, metabolic profile, and lactation performance in Pluriparous dairy cows

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Abstract

This study evaluated the effect of dietary cation–anion balance (DCAB) supplementation during the transition period on postpartum health and productivity in Pluriparous dairy cows. Twelve cows were divided into treatment and control groups under a 60-day pre-partum protocol. The treatment group received ANIONIC MISHRAN from 260 days of gestation until calving. Key parameters including placental expulsion time, incidence of metritis/pyometra, metabolic diseases, calf birth weight, and milk yield were recorded and statistically analysed. Results indicated that cows receiving DCAB supplementation showed significantly faster placental expulsion compared to the control group (4.63 ± 0.33 vs. 6.93 ± 0.22 hours; $p < 0.001$). The treatment group also produced a higher average daily milk yield (9.64 ± 0.16 L/day) than the control group (8.64 ± 0.11 L/day; $p < 0.001$). However, no significant differences were observed between groups in the incidence of metritis/pyometra or calf birth weight. The findings suggest that strategic DCAB supplementation during the transition period can improve key postpartum health parameters and enhance milk production in dairy cows. Therefore, the inclusion of DCAB in pre-partum nutritional management may contribute to improved transition success and greater dairy farm productivity.

Keywords: Dietary cation–anion balance (DCAB), transition period, postpartum health, dairy cows, pluriparous cows

Introduction

India holds the leading position globally in both the production and consumption of milk. In the year 2023–24, dairy cooperatives collected an average of 660 lakh kilograms of milk per day, which was higher than the 589 lakh kilograms per day recorded in 2022–23, reflecting the continued expansion of the dairy sector. The dairy industry is a major component of Indian agriculture, contributing about 5% to the agricultural GDP and nearly 24–25% to the nation's gross domestic product in 2024.

In dairy cattle, the transition phase refers to the period spanning three weeks before calving to three weeks after calving. This stage is regarded as one of the most sensitive and demanding phases in the productive life of dairy animals, requiring careful monitoring and management by farmers. The transition phase plays a significant role in determining post-calving milk yield, the likelihood of metabolic disorders, and the reproductive performance of the animal.

Mechanism behind negative energy balance during the transition period

Effective management during this period is crucial because dairy animals often face an energy deficit due to the increased nutritional requirements associated with the onset

of lactation. Without proper nutritional and management practices during the transition stage, animals may experience reduced productivity in the following lactation cycle. As adequate milk yield is essential for the economic success of dairy farming, ensuring a well-managed transition period is vital for improving productivity, maintaining animal health, and preventing financial losses for dairy producers.

As animals near parturition, their energy requirements rise considerably because of the intense physiological and metabolic changes occurring in the body. During the final stage of pregnancy, the foetus undergoes rapid growth, while the mammary glands develop in preparation for the next lactation. At the same time, the animal works to maintain its energy balance and immune function. If proper nutrition is not provided during this critical period, the animal may develop negative energy balance (NEB). This condition particularly affects high-producing dairy cows, making them more prone to metabolic and infectious disorders such as ketosis, milk fever, mastitis, and metritis, which can result in significant economic losses for the dairy sector.

As calving approaches, hormonal changes occur in which progesterone levels decline while estrogen levels increase. These hormonal shifts often lead to a reduction in dry matter

intake (DMI) of up to 30%. At the same time, the animal's requirement for energy, minerals, and other nutrients increases to support foetal growth, colostrum formation, and milk synthesis. The combination of higher nutrient demand and reduced feed intake contributes to the occurrence of NEB.

During the transition period, several metabolic and endocrine adjustments also take place. Growth hormone activity increases during lactation, stimulating hepatic gluconeogenesis to enhance glucose production and causing insulin resistance. This process reduces glucose utilization by tissues such as the liver, muscles, and adipose tissue, thereby ensuring a greater supply of glucose for milk production. In addition, this metabolic shift promotes lipolysis, releasing non-esterified fatty acids (NEFA) from body fat reserves to support lactogenesis.

Although this is a normal adaptive response in dairy animals, high-yielding cows may experience hypoglycaemia when glucose levels become insufficient. Under such conditions, NEFA undergo partial or incomplete oxidation, resulting in the formation of ketone bodies, including acetone, acetoacetic acid, and mainly β -hydroxybutyric acid (BHBA). Elevated levels of NEFA and BHBA can adversely affect animal health by reducing milk yield, lowering feed intake, weakening immune response, and increasing the risk of metabolic diseases such as ketosis, milk fever, mastitis, and reproductive disorders (Wankhade *et al.*, 2017) [13].

Both NEFA and BHBA serve as important biomarkers of negative energy balance in dairy animals. Higher pre-partum NEFA levels and increased post-partum BHBA concentrations are strongly associated with the development of clinical diseases. In particular, elevated post-partum serum NEFA concentrations are closely linked with a greater risk of conditions such as displaced abomasum, clinical ketosis, metritis, and retained placenta. Research has also shown that cows affected by post-partum disorders generally exhibit higher serum NEFA concentrations and lower plasma insulin-like growth factor-I (IGF-I) levels during the pre-partum period.

Management of transition period

Monitoring Dry Matter Intake

Feed intake usually declines as animals approach parturition, therefore it is important to carefully monitor and manage the energy and nutrient supply of the animal during this stage. Since the quantity of feed consumed becomes lower, the diet should be adjusted so that the animal still receives adequate nutrition. Feeds that are high in energy and nutrients should be offered because insufficient energy intake may result in ketosis in dairy cows. Health problems associated with negative energy balance (NEB) can also reduce fertility performance and may lead to a higher culling rate in dairy herds.

Three-Week Close-Up Period

Dry cows should be shifted to a close-up group approximately three weeks before their expected calving date. This period allows the animal to prepare for lactation by mobilizing calcium from the bones and improving calcium absorption from the intestine, which is essential for proper milk production after calving. The cow also requires

time to gradually adjust to the forage-based and energy-rich diets that will be provided during the lactation phase.

During the dry period, calcium mobilization is relatively low compared with the lactation period, therefore the cow needs time to physiologically adapt to the sudden increase in calcium demand. This adaptation process generally takes 10–15 days, making the close-up period important to prevent health problems after calving.

In the dry period, the ration mainly consists of forages, which form about 70–80% of the diet, to maintain rumen fill. However, such diets are usually low in energy, so grains are gradually introduced during the close-up stage. Depending on the type and quality of forage, about 3–8 pounds of grains can be added to the ration. In addition, supplements such as anionic salts, vitamin E, and selenium (Se) may help strengthen the immune system and reduce the occurrence of post-partum diseases.

Balanced Diet during the Transition Period:

Providing a properly balanced ration during the transition or peri-parturient period is essential to lower the risk of metabolic disorders. A balanced diet should contain adequate levels of energy, protein, carbohydrates, minerals, trace elements, and an appropriate dietary cation–anion difference (DCAD) (Praveen and Dhaarani, 2018) [9]. DCAD refers to the difference between the dietary cations (Na^+ and K^+) and anions (Cl^- and S^{2-}) present in the feed.

Around the time of calving, animals may experience complications such as dystocia, retained placenta, uterine prolapse, and cervical injuries. To minimize these metabolic and post-partum problems, cation–anion supplementation can be included in the diet. Therefore, the present study was conducted to evaluate the effect of anion–cation supplementation during the transition period in Pluriparous cows. The supplement used in this research was ANIONIC MISHRAN, developed by the National Dairy Research Institute (NDRI), Karnal.

Materials and Methods

The cows selected for the study were randomly divided into two equal groups, namely the treatment group and the control group. Animals in Group A (treatment group) were administered ANIONIC MISHRAN orally starting from the 250th day of gestation and continued daily for 21 days. During the experimental period, all cows were maintained on roughage to concentrate ratio of 60:40, consisting of 40% silage and 60% green fodder, along with compound cattle feed provided as the concentrate portion. Cows in Group B were not given the anion–cation supplement and therefore served as the control group. Approximately one week before the expected calving date, all animals were moved to separate calving pens to facilitate better supervision and care.

Immediately after calving, the birth weight of each calf was recorded. Following parturition, the cows were transferred to the milking shed, where their daily milk yield was recorded for a period of three months. Proper oestrus detection methods were employed, and the cows were artificially inseminated (AI) around 60 days after calving. All animals were clinically examined on a daily basis for four months after parturition to allow early detection of any health problems, particularly clinical mastitis, metritis, and

pyometra. The parameters evaluated in this study included the incidence of metabolic disorders, reproductive performance, postpartum health status, milk yield during lactation, and calf body weight at birth and at weaning.

For data evaluation, statistical analyses were carried out using an unpaired t-test for continuous variables and

Fisher's Exact Test for categorical variables to assess the impact of anion-cation supplementation during the transition period. All statistical analyses were performed using the SPSS software package.

Results and Discussion

Parameter (Unit)	Treatment group (Mean SEM)	Control group	P-value	Significance
Placental expulsion time (hrs.)	4.63±0.33	6.93± 0.22	< 0.001	P<0.001
Calf birth weight (kg)	24.48 ±0.32	24.67 ±0.38	0.705	Ns
Calf weaning weight (Kg)	56.08 ±0.78	56.82± 0.58	0.474	Ns
Lactation yield (L/day)	9.64 ±0.16	8.64± 0.11	< 0.001	P<0.001
Metritis/Pyometra incidence (%)	0.0% (0/6)	33.3% (2/6)	0.211*	Ns
Downer cow/Milk fever incidence%	0.0% (0/6)	33.3% (2/6)	0.211*	Ns

*SEM: Standard Error of the Mean. Analyzed by Fisher's Exact Test (two-tailed)

Cows in the treatment group did not show any incidence of metabolic disorders (0%), whereas cows in the control group showed about 33.33% occurrence of milk fever after parturition. The common clinical signs of milk fever include weakness, recumbency, shock, and sometimes death. This condition generally occurs in cows between 5–10 years of age and is most frequently observed between the third and seventh parity (Singh and Singh, 2017) ^[11]. During the peri-parturient period, the calcium requirement of cows increases substantially because of colostrum production, which predisposes multiparous lactating cows to milk fever if adequate physiological adaptation does not occur. Although hypocalcaemia is usually less common in primiparous cows, a study conducted on 115 German herds reported a comparatively higher prevalence of 5.7%. Since the mechanisms responsible for restoring calcium balance require at least 48 hours to become fully functional, milk fever commonly develops within the first 48 hours after calving (Alshehhi *et al.*, 2024) ^[12].

Dietary supplementation had a statistically significant and beneficial effect on two major parameters. The cows receiving supplementation expelled the placenta much earlier (4.63 ± 0.33 hours) compared with the control group (6.93 ± 0.22 hours). This significant reduction ($p < 0.001$) indicates improved postpartum uterine health and a smoother transition after calving. At the same time, cows in the treatment group produced significantly higher milk yield (9.64 ± 0.16 L/day) than the control cows (8.64 ± 0.11 L/day), demonstrating a clear positive influence of anion-cation balance on milk production performance ($p < 0.001$). In contrast, some of the other observations were not statistically significant. No cases of metritis or pyometra were recorded in the treatment group, while two cases (33.3%) occurred in the control group; however, this difference was not statistically significant ($p = 0.211$) due to the limited sample size. Nevertheless, this pattern indicates a possible protective role of supplementation against uterine infections, which should be further evaluated in studies with larger populations. After calving, the foetal membranes remaining in the uterus begin to deteriorate within 6–9 hours, creating a favourable environment for bacterial growth. These bacteria may initially cause acute puerperal metritis, which can later develop into clinical or subclinical endometritis, leading to delayed uterine involution and slower regression of the ovarian corpus luteum after calving (Mordak and Antony, 2015) ^[18].

Furthermore, supplementation did not significantly

influence calf birth weight or weaning weight ($p > 0.05$). This indicates that the benefits of the treatment were mainly maternal, improving the cow's metabolic condition, calving process, and milk production without directly affecting foetal development or postnatal calf growth under the existing management conditions. Overall, the statistical analysis demonstrated that anion-cation supplementation during the transition period in Pluriparous cows significantly reduced the time required for placental expulsion and increased daily milk yield. A noticeable but statistically non-significant trend toward lower uterine infection rates was also observed. However, the supplementation had no measurable effect on calf birth or weaning weights. These findings highlight the importance of appropriate nutritional management during the transition period for improving postpartum health and productivity in dairy cows.

The present study further demonstrates that manipulation of dietary cation-anion balance (DCAB) during the close-up transition period has a significant positive influence on postpartum health and production performance in Pluriparous cows. The results support the hypothesis that optimizing pre-partum nutrition helps reduce the negative effects of negative energy balance (NEB) and hypocalcaemia, which are common challenges during the peri-parturient period (Wankhade *et al.*, 2017) ^[13]. One of the most effective methods to monitor and prevent hypocalcaemia is feeding cows an acidogenic diet during the three weeks preceding calving (Tufarelli *et al.*, 2024) ^[12].

The most prominent outcome of the study was the significant reduction in the time required for placental expulsion. Retained placenta is commonly associated with impaired uterine function and reduced immunity, often linked with hypocalcaemia and inflammatory responses during calving. A properly balanced DCAD diet induces mild metabolic acidosis, which improves calcium mobilization from bones and enhances intestinal absorption of calcium, thereby lowering the risk of subclinical hypocalcaemia (Praveen and Dhaarani, 2018) ^[9]. The faster placental expulsion observed in the treatment group (4.63 vs. 6.93 hours) clearly indicates better calcium balance and a smoother calving process, which aligns with the primary objective of transition cow management. Similar findings have been reported in earlier studies where anionic salt supplementation reduced the incidence of retained foetal membranes (Goff, 2008) ^[4]. Although the occurrence of

metritis or pyometra was not statistically significant, the absence of cases in the treatment group compared with 33.3% in the control group suggests a meaningful biological trend toward improved uterine health. This observation supports the known relationship between metabolic stress, reduced immunity, and postpartum infections (LeBlanc, 2010) [6]. A larger-scale study would be necessary to confirm this protective effect.

At the same time, cows receiving supplementation produced significantly higher daily milk yield (9.64 vs. 8.64 L/day; $p < 0.001$), representing an 11.6% increase in milk production, which has considerable economic importance. This improvement can be attributed to the better metabolic status of cows at calving. By reducing the severity of NEB and hypocalcaemia, DCAB supplementation likely enhanced postpartum feed intake, improved the onset of lactation, and increased the efficiency of nutrient utilization for milk synthesis. These results support the concept that investing in proper transition cow nutrition can directly improve lactation performance, since optimal calcium status strongly influences dry matter intake in fresh cows (Horst *et al.*, 2024; McArt, 2020) [5, 7]. Similar observations were reported by Ghasemi *et al.* (2024) [3], where cows in the control group were 6.43 times more likely to develop metritis compared with normo-calcaemic cows ($p = 0.002$; 95% CI = 1.51–21.56), whereas cows in the treatment group did not differ significantly from normo-calcaemic cows ($p = 0.22$; 95% CI = 0.56–6.52).

Importantly, the positive effects of supplementation were limited to the cows themselves. The absence of significant differences in calf birth weight and weaning weight indicates that the dietary intervention did not influence foetal growth or calf development after birth under the given management conditions. This suggests that the primary action of DCAB supplementation occurs through the regulation of the dam's metabolism and calcium homeostasis, rather than through direct nutritional effects on the foetus or milk composition. This finding agrees with previous studies indicating that DCAB manipulation mainly targets maternal physiology to prevent disorders such as milk fever (Goff, 2008; Rodríguez, 2017) [4, 10]. Similarly, Tereso *et al.* (2011) reported that providing 40–50 g of magnesium (about 0.30–0.45% of dietary dry matter) helps reduce the incidence of hypocalcaemia and hypomagnesaemia in Pluriparous cows.

Conclusion

In conclusion, the strategic supplementation of ANIONIC MISHRAN during the three-week pre-partum period in Pluriparous cows resulted in faster placental expulsion and increased milk production, confirming its effectiveness in promoting a healthier transition period and improved productivity. These findings strongly support the adoption of DCAB-based nutritional management as a routine practice to reduce postpartum complications and enhance the economic sustainability of dairy farming systems. Future studies should focus on evaluating metabolic biomarkers such as NEFA, BHBA, and calcium levels to better understand the physiological mechanisms underlying these beneficial effects.

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