Indian J Dairy Sci 77(3): 258-266
https://doi.org/10.33785/IJDS.2024.v77i03.010

RESEARCH ARTICLE

Anionic mixture supplementation and their impact on hemato-biochemical parameters and post-partum performance of buffaloes under field conditions

Sohan Vir Singh[1](✉), Gaurav Kumar[2] and Rajkumar[3]

Received: 17 October 2023 / Accepted: 15 November 2023 / Published online: 23 June 2024

Abstract: In order to demonstrate the beneficial effect of anionic mixture supplementation to the famers, advanced pregnant (nine months) Murrah buffaloes were selected from adopted village (Kathura, Distt. Sonipat) under Farmer FIRST project. Experimental animals were supplementing anionic mixture (@100gm/day/head) from 21 days before expected date of parturition. Blood samples were collected from the control and experimental group of animals at the start of supplementation of the anionic mixture and after 21 days of parturition. Blood samples were analyzed for hematological and biochemical parameters. Experimental animals were also monitored for the udder development, milk fever, retention of placenta and body condition. The overall mean values of liver enzymes i.e. ALT, AST were numerically lower while the mean values of total protein, Albumin, Globulin, Triglyceride and Potassium were higher during postpartum compared to prepartum period in supplemented than control group. The overall mean values of hematological parameters (Hb and PCV) were found significantly ($P<0.05$) higher in supplemented group than control group during postpartum, whereas no significant difference was observed in RBC and WBC concentration among control and treatment group. Based on the farmer's feedback and physical observations, the development of the udder size was more prominent in supplemented group than control. No case of milk fever was observed in supplemented group of buffaloes. Timely expulsion of placenta was significantly ($P<0.05$) higher in supplemented group of buffaloes than control. Significantly ($P<0.05$) higher milk yield was recorded in supplemented group of buffaloes than control. Glossy skin, shiny hair coat and increase in resistance of diseases were also reported in treatment group of buffaloes. Based on the results of the study, it can be stated that supplementation of anionic mixture is helpful in improving the health and reproductive status of buffaloes.

Keywords: Anionic mixture; Buffaloes; Hemato-biochemical; Murrah; Reproduction

[1]ICAR-National Dairy Research Institute, Karnal, Haryana 132 001 India

[2]ICAR-CSSRI, Karnal -132001

(✉)Animal Physiology Division, ICAR-National Dairy Research Institute, Karnal- 132001.
Email: Sohanvir.singh@icar.gov.in

Introduction

The productive and reproductive performance of dairy animals is influenced by several environmental factors, such as climate and geography (García et al. 2007). Peripartum disorders negatively influences productive and reproductive performance of dairy animals. Therefore, it is important to consider the characteristics and farming background of that area. Giuliodori *et al.* (2013) reported that peripartum management influences subsequent reproductive performance, which is one of the most important factors influencing efficient dairy management. Milk fever, a metabolic disease, and retention of placenta affects high producing dairy animals just after calving are causing the reduction in milk yield. The major reasons for these diseases are low blood calcium levels in these animals. The Incidence of retention of placenta and milk fever varies 4-5% during second lactation and increases with increasing age. Therefore, there is an economic loss due to investment on veterinary services, medicines and reduction in milk yield. The transition from gestation to lactation requires physiological adaptations by the dairy animals, which can significantly affect the following lactation and subsequent reproduction. Nutrition management during the transition period is challenged by reduced DMI during the late gestation period coupled with a drastic increase in nutrient requirements after calving. One of the most significant challenges involves calcium homeostasis and can result in clinical or subclinical hypocalcaemia. Block (1984) reported that cows experiencing clinical hypocalcaemia during the immediate periparturient period produced 14% less milk than cows with normal serum calcium concentrations. In addition to decreased milk yield, cows that experienced clinical or subclinical hypocalcaemia are at greater risk for developing other metabolic disorders (Curtis et al. 1984). Feeding negative DCAD diets

prepartum stimulated calcium absorption and mobilization, thus preventing hypocalcaemia and maintained DMI and improved the post partum milk yield (Block, 1984). Therefore, proper feeding and management during the transitional period may improve postpartum productivity and reproductive performance of dairy cows. Additionally, the incidence of common clinical diseases is closely associated with calving, with the high-risk period being within 21 days after calving. Periparturient period (from 3 weeks before parturition to 3 weeks after parturition) is associated with multiple changes including hormonal and biochemical changes, serum levels of major macro minerals etc. (Singh, 2014). In this regard, metabolic profiling, which refers to the analysis of blood biochemical constituents, is an important tool for detecting metabolic disorders in dairy animals before any clinical manifestations appear (Baèiæ et al. 2007 and Rossato et al. 2001). Naher et al. (2020) reported 30% subclinical hypocalcaemia and 25% subclinical ketosis in crossbred lactating cows. Furthermore, it is important to have baseline data on actual serum concentration of different hemato-biochemical and mineral status during periparturient period that will certainly help to prevent metabolic diseases. This study, therefore, was conducted to study the effect of anionic mixture supplementation on hemato- biochemical parameters, mineral status, incidence of milk fever, retention of placenta, milk yield etc during periparturient period in buffaloes under field conditions.

Materials and Methods

Thirty six pregnant Murrah buffaloes (2-3 parity) were selected from the adopted village (Kathura) of Sonipat District under the Farmer FIRST project and equally divided into two groups i.e. control and treatment. Treatment group of animals were started supplementing Anionic Mixture (@100 gm/day/animal before 21 days of expected date of calving and supplemented till the day of calving. All the experimental animals were fed traditionally as per the availability of feed and fodders at farmer's door step and concentrate mixture @ 3kg/day/animal were also fed. Blood samples from all the experimental animals were collected 21 days before expected date of calving (just before supplementation) and after 21 days of calving. Immediately after collection, blood samples were transported to laboratory at ICAR-NDRI, Karnal in an ice box for hematological and biochemical parameters. Blood samples were centrifuged and blood plasma was separated and stored at -20°C for further analysis of biochemical parameters.

Hematological parameters i.e. red blood corpuscles (RBC), hemoglobin (Hb) and packed cells volume (PCV) was estimated using the standard methods.

Biochemical Parameters

Total plasma protein

Total plasma proteins were estimated using commercial diagnostic kit of Recombigen Laboratories Pvt. Ltd., New Delhi.

Assay principle: The peptide bonds of proteins react with cupric ions alkaline solution to form a colored chelate, the absorbance of which was measured at 578 nm (550-580 nm). The biuret reagent contains sodium-potassium tartrate, which helped in maintaining solubility of this complex at alkaline pH.

Calculation:

$$\text{Total Protein concentration (g/dL)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 6$$

Plasma albumin

Plasma albumin was estimated using commercial diagnostic kit purchased from (Recombigen Laboratories Pvt. Ltd., New Delhi).

Assay principle: Plasma albumin in presence of Bromocresol-Green (BCG) under acidic condition forms green colored complex whose absorbance read at 630nm wavelength. Absorbance at this wavelength was proportional to the albumin concentration in plasma.

Calculation:

$$\text{Albumin (gm/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 3$$

Plasma Globulin

Globulin was calculated using following formula:

Globulin=Total protein - Albumin

Liver enzymes

Aspartate aminotransferase and Alanine aminotransferase was estimated using commercial diagnostic kit (Recombigen Laboratories Pvt. Ltd., New Delhi).

Estimation of minerals in blood plasma

The concentration of minerals (calcium, phosphorus and sodium) in blood plasma was estimated using atomic absorbance spectrometer (AA7000F) in Central laboratory of ICAR- CSSRI, Karnal, Haryana.

For preparation of mineral extract, 1 ml plasma sample was taken and 5 ml of tri-acid mixture was added. The samples were digested using Kelplus- KES 12L R digestion system (Pelican India Ltd., Chennai, India). When the digested samples became clear, the tubes were taken out and the samples were allowed to cool. To the digested samples, a few ml of doubled distilled water (DDW) was added and passed through Whatman filter paper No. 42 and final volume was made to 10 ml. Standard curves were prepared for different minerals. The samples were run under same

conditions and concentration of a particular mineral in samples was determined using standard curve.

***Calcium*:** Absorbance of standard and test against blank was recorded at 630 nm (600-650).

Sodium and phosphorus: Absorbance of standard and test against blank was recorded at 589 nm and 766 nm respectively

All the experimental animals were monitored for their health, body condition score and udder development regularly throughout the study period. After calving milk fever, retention of placenta daily milk yield and their composition were also monitored.

The data was statistically analyzed for mean and standard error and significance using SPSS software (version 26.0).

Ethical approval

The experiment was approved and carried out according to the established standards of the Institutional Animal Ethics Committee (IAEC) which was formed in accordance with Article 13 of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) rules ((IAEC Approval No. 42-IAEC-18-7).

Results and Discussion

Hematological parameters

Red and white blood corpuscles

The results of the hematological parameters i.e. red blood corpuscles (RBC), white blood corpuscles (WBC), hemoglobin (gm %) and packed cell volume (PCV%) have been presented in fig.1. The RBC varied from 6.88 to 8.68 millions/ µl with an overall mean of 7.98 ± 0.16 millions /µl in control group of pregnant buffaloes. The respective values of RBC in treatment group of pregnant buffaloes varied from 6.24 to 9.29 millions/µl with an overall mean values 7.84 ± 0.25 millions/µl, 21 days before the expected date of calving. On 21 days postpartum, the RBC varied from 7.03 to 9.19 millions/µl with an overall mean values 7.84 ± 0.17 millions/ µl in control group of buffaloes whereas the respective values of RBC in treatment group of buffaloes varied from 7.04-9.72 millions/µl with an overall mean values 8.37 ± 0.23 millions/µl (Fig.1). The mean values of RBC found during the present study are within the normal range (5.12-8.54 millions/ µl) as reported by Dhillon et al. (2020) in buffaloes.

The WBC varied from 9.53 to 12.32 thousands/ µl with an overall mean of 10.39 ± 0.22 thousands /µl in control group of pregnant buffaloes. The respective values of WBC in treatment group of pregnant buffaloes varied from 7.70 to 15.30 thousands/ µl with an overall mean values 10.35 ± 0.49 µl, on 21 days before the expected date of calving. On 21 days postpartum, the WBC varied from 8.90 to 13.90 thousands/ µl with an overall mean values 11.11 ± 0.35 thousands/ µl in control group of buffaloes whereas the respective values of WBC in treatment group of buffaloes varied from 7.20-13.40 thousands/ µl with an overall mean values 10.17 ± 0.41 thousands/ µl. Kour et al. (2023) also reported significantly ($p<0.05$) higher values of WBC ($x10^3/\mu l$) during post partum period compared to pre-partum period in buffaloes.

Hemoglobin and Packed cell volume

The overall mean values of Hb and PCV was 11.01±0.28 gm% and 34.82±0.66% respectively during prepartum period (21 days before expected date of calving) in control group of buffaloes. Whereas, the respective values of Hb and PCV was 11.25±0.24 gm% and 35.94±0.77% respectively in treatment group of buffaloes. The Hb and PCV values varied from 10.80 to 12.80 gm% and 31.60 to 42.10 % with an overall mean value of 11.63±0.35 gm% and 35.97±0.86% respectively during prepartum period in control group of buffaloes (Fig.1). Whereas, the respective values of Hb and PCV varied from 11.00 to 15.40 gm% and 37.00 to 51.30 % with an overall mean values of 12.30±0.33 gm% and 40.15±1.18 % respectively during postpartum period in treatment group of buffaloes. Dhillon et al. (2020) also reported almost similar range of Hb from 8.9 to 14.2 gm% in buffaloes

Liver enzymes

Aspartate transaminase (AST):

The AST activity varied from 233.4 to 309.6 IU/ L with an overall activity of 267.52±5.80 IU/ L in control group of pregnant buffaloes. The respective activity of AST in treatment group of pregnant buffaloes varied from 239.9 to 316.6 IU/ L with an overall mean activity 272.65 ± 5.06 IU/ L, on 21 days before the expected date of calving. On 21 days postpartum, the AST activity ranged from 243.6 to 314.7 IU/ L with an overall mean activity 276.22 ± 5.84 IU/ L in control group of buffaloes whereas the respective activity of AST in treatment group of buffaloes varied from 215.7-297.1 IU/ L with an overall mean activity 263.44±6.07 IU/ L (Fig.2) . The activity of AST was found to be significantly ($P<0.05$) lower during post partum period in anionic mixture supplemented group than control. A significant increase ($p<0.05$) in the mean activity of serum glutamic-oxaloacetic transaminase (SGOT) has been observed in buffaloes of prepartum to postpartum period indicating of stressed hepatic metabolism and pronounced catabolism of body reserves (Kour et al. 2022). Similar findings related to levels of SGOT and SGPT were found by Abdulkareem (2013) and Fiore et al. (2017) . The changes in the concentrations of liver enzymes indicate metabolic disorders with involvement of liver even at sub-clinical levels (Fiore *et al.* 2015).

***Alanine aminotransferase* (*ALT*)**

On 21 days before the expected date of calving, the ALT activity varied from 47.62 to 78.92 IU/ L with an overall mean of 61.28±2.55

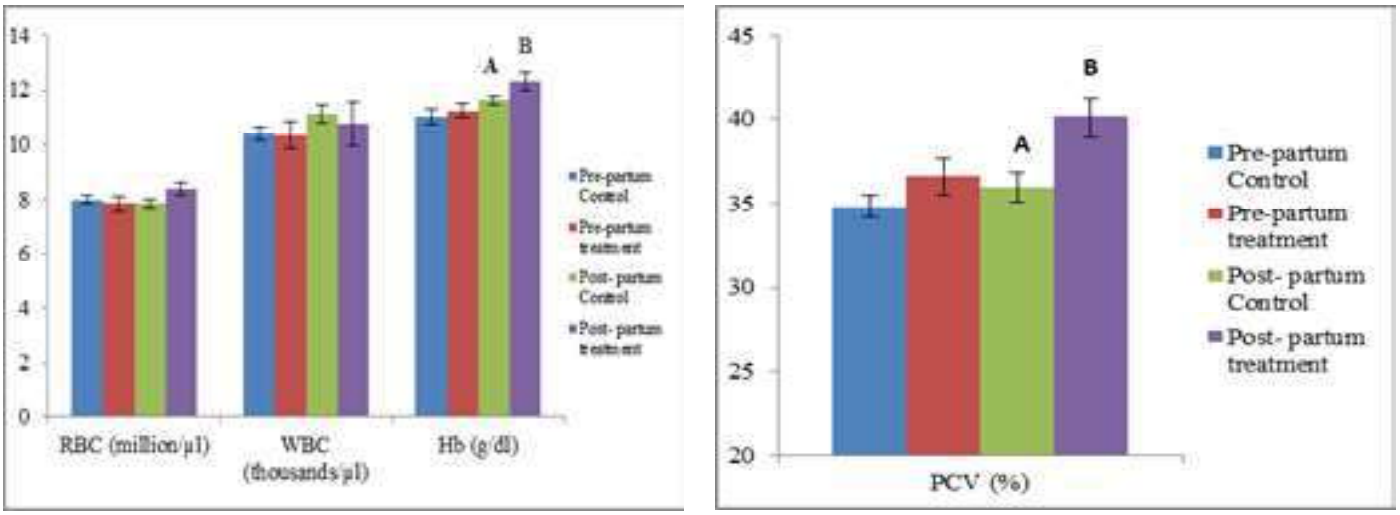


Fig. 1 Effect of anionic mixture supplementation on hematological parameters of periparturient buffaloes

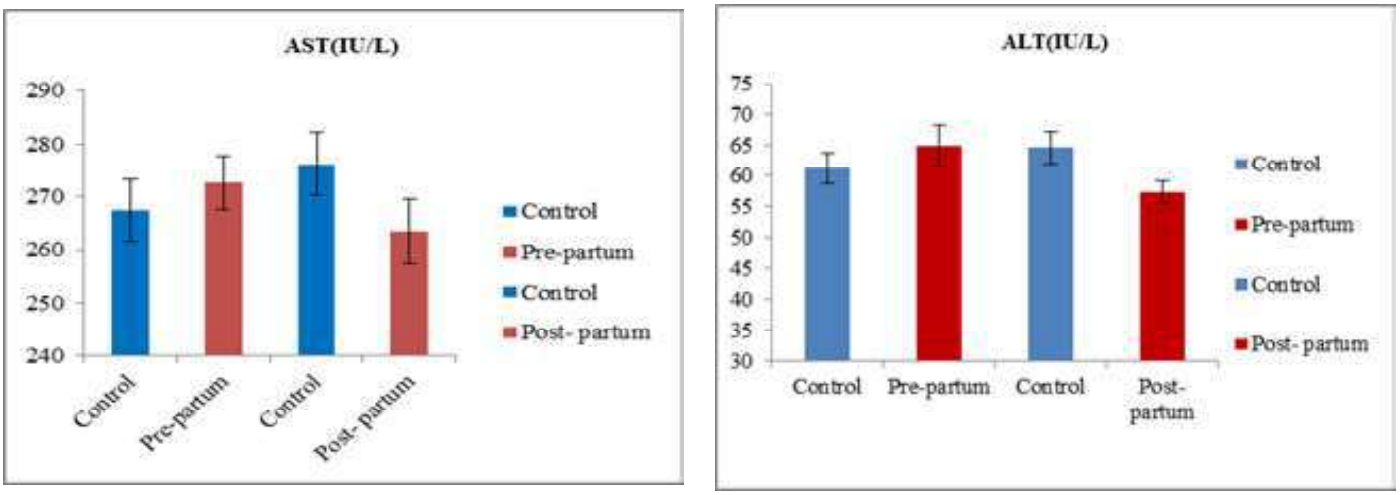


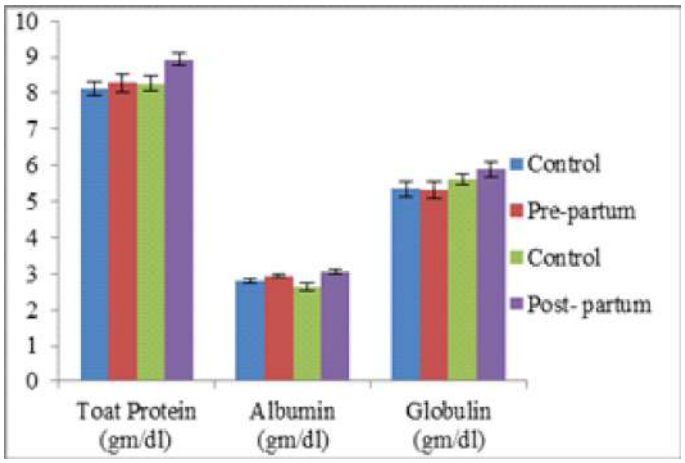
Fig. 2 Effect of anionic mixture supplementation on plasma liver enzymes levels of periparturient buffaloes

IU/ L in control group of pregnant buffaloes. The respective activity of ALT in treatment group of pregnant buffaloes varied from 44.47 to 87.02 IU/ L with overall mean values 64.94±3.41 IU/ L. On 21 days postpartum, the ALT activity varied from 52.64 to 80.54 IU/ L with an overall mean activity of 64.67±2.67 IU/ L in control group of buffaloes whereas the respective activity of ALT in treatment group of buffaloes varied from 43.45-68.62 IU/ L with an overall mean activity of 57.45±1.65 IU/ L (Fig.2) .

The liver as a most important organ in ruminant metabolism and also very sensitive to nutrition fed to the animals. The significant ($P<0.05$) lower activity of AST and ALT in treatment group compared to control group of buffaloes during the present investigation are in accordance with those of Gonzalez et al. (2011) and Kataria and Kataria (2012) who reported that ALT and AST activity in serum/plasma are commonly used as markers of liver damage resulting from metabolic disease or stressors. Higher ALT and AST activities were observed due to the damage of liver, resulting in release of these cellular enzymes into the serum. The ALT and AST activities were increased linearly with decreasing DCAD, suggesting that the cows incurred some degree of liver damage (Gonzalez et al. 2011).

Total Protein

The total protein levels varied from 6.94 to 9.24 g/dl with an overall mean of 8.13±0.19 g/dl in control group of pregnant buffaloes. The respective values of total protein levels in treatment group of pregnant buffaloes varied from 6.44 to 9.66 g/ dl with an overall mean values 8.28±0.24 g/dl, on 21 days before the expected date of calving. On 21 days postpartum, the total protein levels varied from 6.96 to 9.62 g/dl with an overall mean values 8.27±0.22 g/dl in control group of buffaloes whereas the respective values of total protein levels in treatment group of buffaloes varied from 7.50 to 9.97 g/dl with an overall mean values



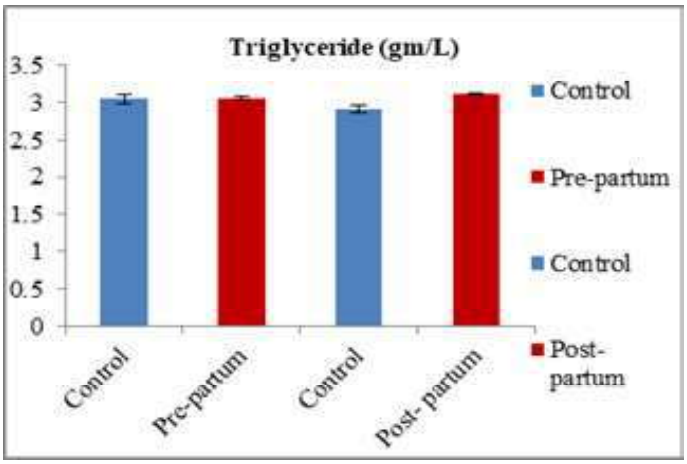
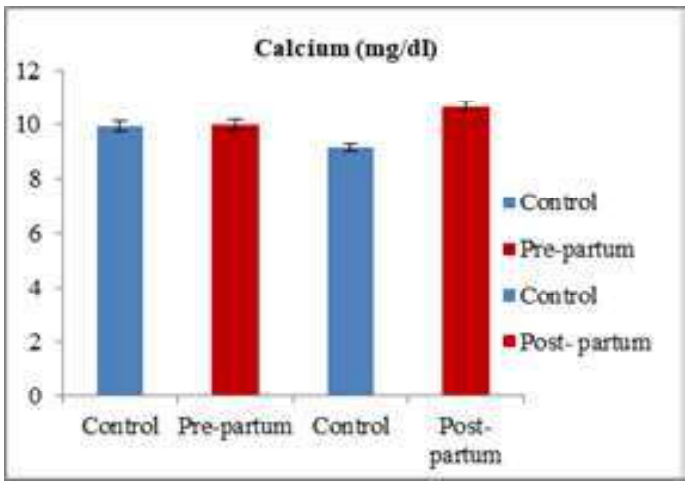
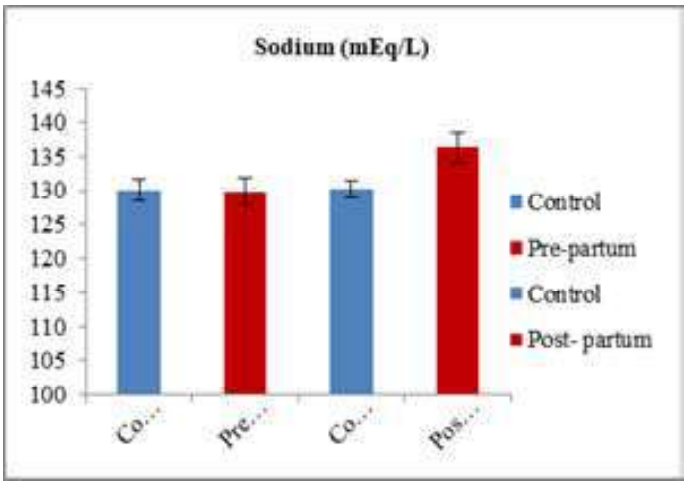


Fig. 3: Effect of anionic mixture supplementation on plasma protein and triglyceride levels of periparturient buffaloes





8.94±0.18 g/dl (Fig.3). The total protein content was non-significantly lower and higher in control and treatment group of buffaloes respectively during post partum than preparation period in buffaloes. Abdulkareem (2013) also found non-significant difference ($p>0.05$) among the mean concentrations of total protein, albumin and globulin in riverine buffaloes during transition period.

Albumin

On 21 days before the expected date of calving, the albumin content varied from 2.17 to 3.19 g/dl with an overall mean of 2.79±0.07 g/dl in control group of pregnant buffaloes. The respective values of albumin content in treatment group of pregnant buffaloes varied from 2.68 to 3.21 g/dl with an overall mean values 2.95±0.05 g/dl. On 21 days postpartum, albumin content varied from 1.97 to 3.28 g/dl with an overall mean values 2.65±0.11 g/dl in control group of buffaloes whereas the respective values of albumin content in treatment group of buffaloes varied from 2.64 to 3.28 g/dl with an overall mean values 3.04±0.06 g/dl (Fig.3) .

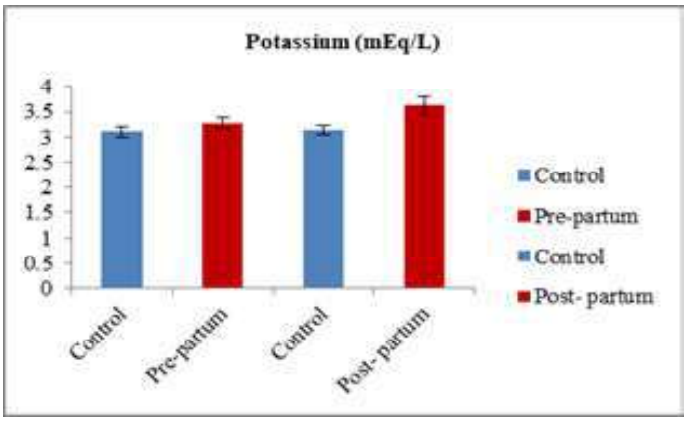


Fig. 4 Effect of anionic mixture supplementation on plasma minerals content of periparturient buffaloes

Globulin

The overall mean values of globulin content were 5.34±0.22 and 5.32±0.23 g/dl in control and treatment group of pregnant buffaloes respectively on 21 days before the expected date of

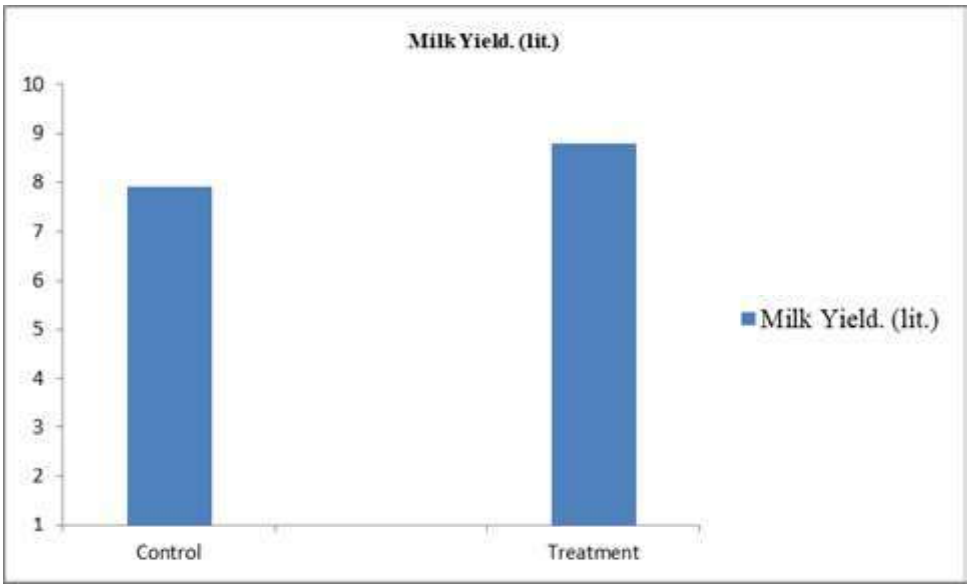


Fig. 5 Effect of anionic mixture supplementation on milk yield of buffaloes

calving. Whereas the respective values of globulin content was 5.61±0.16 and 5.90±0.21 g/dl in control and treatment group of buffaloes respectively during post partum period (Fig.3).

The results obtained during the present investigation i.e. higher albumin content in Anionic mixture supplemented group during post partum period are in accordance with those of Seifi et al. (2005) in dairy cows.

Plasma minerals

Potassium content

Twenty one days before the expected date of calving, the potassium content in blood plasma varied from 2.56 to 4.09 mEq/L with an overall mean value of 3.28±0.10 mEq/L in control group of pregnant buffaloes. The respective values of potassium content in treatment group of pregnant buffaloes varied from 2.59 to 3.80 mEq/L with an overall mean value 3.28±0.10 mEq/L. On 21 days postpartum, potassium content varied from 2.57 to 3.59 mEq/L with an overall mean values 3.23±0.17 mEq/L in control group of buffaloes whereas the respective values of potassium content in treatment group of buffaloes varied from 2.87 to 4.98 mEq/L with an overall mean values 3.63±0.17 mEq/L (Fig.4). No significant difference was observed in potassium content in blood plasma of buffaloes during peripartum period in control and supplemented group. The values of potassium was found during the present investigation are in accordance with those of Dhillon et al. (2020) who reported the reference values of plasma potassium 3.55–5.9 mmol/L in Murrah buffaloes. Mahanrao et al. (2016) also did not find any difference in the potassium content in blood plasma of cows among pre and post partum period. These results of the present study are consistent with those of Tucker et al. (1988b) who observed non-significant effect of altering DCAD levels on plasma K+ concentrations in lactating cows.

Sodium content

Prior to parturition (21 days), the sodium content in blood plasma varied from 123 to 140 mEq/L with an overall mean values of 130.07± 1.59 mEq/L in control group of pregnant buffaloes. The respective values of sodium content in treatment group of pregnant buffaloes varied from 118 to 146 mEq/L with an overall mean values 129.87±2.03 mEq/L (Fig.4). Dhillon et al. (2020) also reported the reference values of plasma sodium of 125.7–164 mmol/L in Murrah buffaloes. On 21 days postpartum, sodium content varied from 123 to 137 mEq/L with an overall mean values 130.27±1.17 meq/L in control group of buffaloes whereas the respective values of sodium content in treatment group of buffaloes varied from 122 to 149 mEq/L with an overall mean values 136.27±2.09 meq/L. The higher plasma sodium content in treatment group of buffaloes during the present study during post partum period are in accordance with those of Mahanrao et al. (2016) who also found positive correlation with dietary DCAD supplemented group and plasma sodium level was found maximum in +31 meq/100 g of DM DCAD fed cows. Tucker et al. (1988b) also reported higher plasma sodium at high DCAD levels. These results are in consistent with previous reports in lactating cows and calves (Tucker et al. (1988b) and buffaloes (Shahzad et al. 2011). The findings of this study are in line with Tucker et al. (1988b) who reported higher plasma sodium at high DCAD levels. The plausible explanation of this might be increased uptake of sodium at high DCAD level. Serum potassium concentration remained unaffected (4.22 to 4.24 meq/L) by DCAD levels.

Calcium content

The calcium content in the blood plasma varied from 8.72 to 11.14 mg/dl on 21 day prior to calving with an overall mean of 9.91±0.20 mg/dl in control group of pregnant buffaloes. The respective values of calcium content in treatment group of pregnant

Photo 1: Effect of anionic mixture supplementation on udder development and body condition of buffaloes under farmer's field

Buffalo without supplementation

Buffalo supplemented with anionic mixture

buffaloes varied from 9.16 to 11.67 mg/dl with an overall mean values 9.98±0.18 mg/dl (Fig.4). On 21 days postpartum, calcium content in the blood plasma varied from 8.29 to 10.06 mg/dl with an overall mean values 9.17±0.14 mg/dl in control group of buffaloes whereas the respective values of calcium content in treatment group of buffaloes varied from 9.49 to 11.88 mg/dl with an overall mean values of 10.66±0.19 mg/dl. The calcium content in blood plasma of supplemented group of buffaloes was significantly ($P<0.05$) higher than control group. The values found during the present study are well within the normal range reported by Dhillon et al. (2020) i.e. 8.72–12.3 mg/dl plasma calcium in Murrah buffaloes. In the periparturient period from advanced pregnancy to early lactation, the clearance of calcium to the placenta ceases, but the lactation calcium demand increases rapidly (Ramberg et al. 1984). The reduction of body fluid's pH by addition of low dietary cation-anion difference (DCAD) diet can help in calcium mobilization (Bushinsky et al. 1993; Schonewille et al. 1994). The results of the present study are in accordance with those of Mahanrao et al. (2016) who reported the higher levels of calcium after parturition in Hariana cows. Blood calcium level was higher for the lowest DCAD (+11 meq/100g of DM), suggesting that low DACD helps in mobilization of stored calcium in periparturient cows. A negative DCAD diet tends to increase the serum calcium, directly by calcium mobilization from bones and indirectly through increased absorption from the intestine due to increased synthesis of 1,25(OH) 2D3 (Block,1984).

Triglycerides (gm/L)

The triglycerides content of blood plasma varied from 2.58 to 3.42 g/dl with an overall mean of 3.04±0.06 g/dl in control group of pregnant buffaloes on 21 day prior to calving. The respective values of triglycerides content of blood plasma in treatment group of pregnant buffaloes varied from 2.81 to 3.11 g/dl with an overall mean values 3.05±0.02 g/dl. The plasma content of triglycerides was almost similar at the start of the experiment. On 21 days postpartum, triglycerides content varied from 2.58 to 3.21 g/dl with an overall mean values 2.90±0.05 g/dl in control group of buffaloes whereas the respective values of triglycerides content in treatment group of buffaloes varied from 2.99 to 3.16 g/dl with an overall mean values 3.11±0.01 g/dl (Fig. 3). The basal values of triglycerides in blood plasma of both the group of buffaloes were higher whereas upper limit remained within the normal physiological range (1.44 – 3.60g/dl) as reported by Mamun et al. (2013) and Cozzi et al. (2011) in cattle.

Reproductive performance

Deficiency of minerals like calcium, magnesium, phosphorus, copper, selenium, zinc, manganese etc. in the blood plasma of dairy animals have been linked with occurrences of milk fever, retained placenta, abortion, dystocia, vaginal prolapse, downer cow syndrome and ultimately reduced reproductive performance (Amen and Muhammad, 2016; Mokolopi, 2019; Yatoo et al. 2018). During the present study, supplementation of anionic mineral mixture to buffaloes enhanced the udder development visibly much faster compared to control group of buffaloes (photo 1). No case of milk fever was reported in anionic mineral mixture supplemented group, whereas two animals from control group showed the symptom of milk fever. The results of Joyce et al. (1997) also showed no case of retained placenta in cows fed with DCAD diets. After parturition, due to higher milk yield and colostrum production in dairy cows and buffaloes, calcium requirement may increase up to 10 times than the dry period (Patel et al. 2011). If this additional requirement of calcium is not fulfilled, calcium deficiency leads to a condition known as hypocalcaemia or milk fever. It is a chronic incident notably observed in high yielding dairy animals. Milk fever is a first step for several other reproductive problems (Buragohain and Kalita 2016). Milk fever is an important production disease occurring most commonly in adult dairy animals within 48-72 hours after parturition, which is characterized clinically by hypocalcemia, general muscular weakness, circulatory collapse and depression of consciousness. Higher risk of mastitis, retained placenta,

dystocia, prolapsed uterus, metritis, delayed uterine involution, retained fetal membranes, displaced abomasum, reduced feed intake, poor rumen and intestine motility was reported in the bovines affected with mastitis (Goff 2008; Oetzel and Miller 2012; Reinhardt et al. 2011). During the present investigation, all the buffaloes of supplemented group expelled the placenta in time whereas three animals from control group took more time for expulsion of placenta. Razzaghi et al. (2012) also reported the similar results. Generally expulsion of placenta ranged from 2.5 to 7 hours. In the present study, placenta expulsion time of buffaloes supplemented with anionic mixture was within reported ranges i.e. around 5 hours.

The health status, body condition score and shining of the skin was improved with supplementation of Anionic mixture compared to control group of buffaloes (photo 1). Milk fever is a nutritional disorder which could be prevented with proper feeding of dairy animals during prepartum. The prepartum supplementation of anionic diets prevent milk fever as reported by several researchers (Shahzad et al. 2008; Charbonneau et al. 2006; Lean et al. 2019; Melendez et al. 2019; Wu et al. 2008).

When milk fever results due to imbalance in blood Ca, P and Mg levels, it is known as "Milk fever complex". Generally the milk fever is sporadic but on individual farms the incidence may rarely reach 25-30% of susceptible cows and increases with age and yield. During the present investigation no case of milk fever was observed in the anionic supplemented group of buffaloes.

Milk Yield

The supplementation of anionic mixture is economical for dairy farmers which improves the milk yield (by 10%), fat content of the milk and the immunity of the animals apart from preventing various diseases (Charbonneau et al. 2006). The milk yield of control and anionic mixture supplemented group was recorded weekly and data has been presented in Fig. 5. The average milk yield was 7.90±0.11 litres/day (ranged 6.5 to 9.5 litres/day) and 8.80±0.11 litres/day (7.25 to 10.30 litres/day) in control and supplemented group of buffaloes respectively. Supplementation of anionic mixture prior to 21 days of expected date of calving to buffaloes increased the milk yield by 10.13 % over the control group. Milk yield was found significantly higher ($P<0.05$) in treatment than control group of buffaloes. Cariappa et al. (2021) reported 12% increase in milk yield of buffaloes under field conditions by supplementation of anionic mixture. Tucker et al. (1988b) also reported 9% increase in milk yield of early lactating cows. Moore et al. (2000) also reported that feeding of negative DCAD to periparturient dairy cows proved a useful nutritional practice; it enhanced blood calcium and postpartum milk production. Several other authors also demonstrated that cows fed higher DCAD level produced more milk during early lactation (Hu and Murphy, 2004; Hu et al. 2007a). The potential effect of DCAD on lactating dairy cows has also been explored, and results indicate that DCAD and production are related possibly through acid-base regulation (Hu and Murphy, 2004).

Conclusion

The supplication of anionic mixture to advanced pregnant buffaloes improved the hematological, plasma protein and their fractions, mineral status and lowered the levels of liver enzymes during postpartum compared to prepartum period in supplemented than control group. Udder size was more prominent and no case of milk fever was observed in supplemented group. Higher ($P<0.05$) milk yield was recorded in supplemented group of buffaloes than control. Glossy skin, shiny hair coat and increase in resistance of diseases were also reported in treatment group of buffaloes. Based on the results of the study, it can be stated that supplementation of anionic mixture is helpful in improving the health and reproductive status of buffaloes.

Acknowledgements

The authors would like to thank the Director of ICAR-NDRI, Karnal and Director, ICAR-CSSRI, Karnal for providing the facilities for carrying out the research work. Fund received from ICAR, New Delhi under Farmer FIRST project is highly acknowledged.

References

Abdulkareem TA (2013) Some hematological and blood biochemical attributes of Iraqi riverine buffaloes (*Bubalus bubalis*) around calving and postpartum periods. Al-Anbar J Vet Sci 6: 143-150

Amen MHM, Muhammad SS (2016) Effect of zinc supplementation on some physiological and growth traits in local male rabbit. Vet J 6: 151–155

Baèiæ G, Karadjole T, Maèešiæ N, Karadjole M (2007) A brief review of etiology and nutritional prevention of metabolic disorders in dairy cattle. Vet *arhiv* **77:**567–77

Block E (1984) Manipulating dietary anions and cations for prepartum dairy cows to reduce incidence of milk fever. J Dairy Sci 67:2939-2948

Buragohain R, Kalita G (2016) Milk fever: Prevention strategies with special emphasis on nutritional management. CAU Farm Mag 6: 41–43

Bushinsky DA, Wolbach W, Sessler N, Mogilevsky R, Levi-Setti R (1993) Physicochemical effects of acidosis on bone calcium flux and surface ion composition. J Bone Min Res 8: 93–102

Cariappa A A, Chande BS, Sankhala G, Mani V, Sendhil R, Dixit A, Meena B S (2021) Estimation of economic losses due to milk fever and efficiency gains if prevented: evidence from Haryana, India. https://arxiv.org/abs/2105.09782

Charbonneau E, Pellerin D, Oetzel GR (2006) Impact of Lowering Dietary Cation-Anion Difference in Nonlactating Dairy Cows/ : A Meta-Analysis. J Dairy Sci 89: 537–548.

Cozzi G, Ravarotto L, Gottardo F, Stefani AL, Contiero B, Moro L, Brscic, M, Dalvit P (2011) Short communication: Reference values for blood parameters in Holstein dairy cows: Effects of parity, stage of lactation, and season of production. J Dairy Sci 94: 3895–3901

Curtis CR, Erb HN, Sniffen CJ, Smith RD (1984) Epidemiology of parturient paresis: predisposing factors with emphasis on dry cow feeding and management. J Dairy Sci 67:817-825

Dhillon KS, Randhawa CS, Gupta K, Singh RS, Chhabra S (2020) Reference values for haematological and biochemical profile in adult Indian buffaloes. Buffalo Bull 39:145–154.

Fiore E, Barberio A, Morgante M, Rizzo M, Giudice E, Piccione G, Lora M, Gianesella M (2015) Glucose infusion response to some biochemical parameters in dairy cows during the transition period. Animal Sci Papers and Reports 33(2): 129-136.

Fiore E, Giambelluca S, Morgante M, Contiero B, Mazzotta E, Vecchio D, Vazzana I, Rossi P, Arfuso F, Piccione G, Gianesella M (2017) Changes in some blood parameters, milk composition and yield of buffaloes (*Bubalus bubalis*) during the transition period. Anim Sc J 88: 2025-2032.

García-Ispierto I, López-Gatius F, Santolaria P, Yániz JL, Nogareda C, López-Béjar M (2007) Factors affecting the fertility of high producing dairy herds in northeastern Spain. *Therio* 67**:** 632–638

Giuliodori MJ, Magnasco RP, Becu-Villalobos D, Lacau-Mengido IM, Risco CA, de la Sota RL (2013) Clinical endometritis in an Argentinean herd of dairy cows: risk factors and reproductive efficiency. J Dairy Sci 96**:** 210–218

Goff JP (2008) The monitoring, prevention, and treatment of milk fever and subclinical hypocalcemia in dairy cows. Vet J 176: 50–57

Gonzalez FD, Muino R, Pereira V, Campos R, Benedito JL (2011) Relationship among blood indicators of lipomobilization and hepatic function during early lactation in high-yielding dairy cows. J Vet Sci 12:251–255

Hu W, Murphy M R (2004) Dietary cation anion difference effects on performance and acid base status of lactating dairy cows: a meta-analysis. J Dairy Sci 87: 2222 –2229

Hu W, Murphy MR, Constable PD, Block E (2007a) Dietary cation-anion difference and dietary protein effects on performance and acid-base status of dairy cows in early lactation. J Dairy Sci 90:3355–3366

Joyce PW, Joyce WK, Sanchez JP, Goff (1997) Effect of anionic salts in prepartum diets based on alfalfa. J Dairy Sci 80: 2866-2875

Kataria N, Kataria AK (2012) Use of serum gamma glutamyl transferase as a biomarker of stress and metabolic dysfunctions in *Rathi* cattle of arid tract in India. J Stress Physiol and Biochem 8:23–29

Kour S, Sharma N, Kour S (2022).Transition period in dairy animals; physiological and molecular insights. Transition. 10: 237-246.

Kour S, Sharma N, Zul-I-Huma, Ahemd T, Kour S, Pathak AK (2023) Haemato-biochemical Profiling in Buffaloes in Relation to Metabolic Changes during Transition Period. Indian J Anim Res doi10.18805/IJAR.B-4992

Lean IJ, Santos JEP, Block E, Golder HM (2019) Effects of prepartum dietary cation-anion difference intake on production and health of dairy cows/ : A meta-analysis. J Dairy Sci 102: 1–31

Mamun M, Hassan M, Shaikat A, Islam S, Hoque M, Uddin M, Hossain M B (2013) Biochemical analysis of blood of native cattle in the hilly area of Bangladesh. Bangladesh J Vet Med 11**:** 51–56

Melendez P, Zaror V, Gaul P, Poock S E, Goff J P (2019) Effect of diets containing sulfate or chloride-based anionic salts, fed to grazing prepartum dairy cows, on concentrations of Ca in plasma, disease incidence and milk yield. New Zealand Vet J 67**:** 79–85

Mohanrao B A, Kumar V, Roy D, Kumar M, Srivastava M, Gupta V P (2016) Influence of dietary cation-anionic difference on hematobiochemical profile, mineral metabolism, post-partum reproductive and productive performance of Hariana cows. Indian J Anim Res 50: 497-504

Mokolopi B G (2019) Phosphorus, calcium, and magnesium contents of pasture and their effect on body condition scores and body mass of communal cattle depending on natural pasture of Mogosane Village, of the North West Province, South Africa. J Trop Animal Hlth and Prod 51**:** 1295–1787

Moore SJ, Vandehaar MJ, Sharma K, Pilbcam TF, Beede DK, Bucholtz F, Liesman JS, Horst RL, Goff JP (2000) Effect of altering dietary cation-Anion difference on calcium and energy metabolism in peripartum cows. J Dairy Sci 83:2095–2104.

Naher L, Samad M, Siddiki S, Islam M (2020) Prevalence and risk factors of subclinical milk fever and ketosis in lactating cross-bred dairy cows with their therapeutic management in Bangladesh. J Vet Med Res 2:139–182

Oetzel GR, Miller BE (2012) Effect of oral calcium bolus supplementation on early-lactation health and milk yield in commercial dairy herds. J Dairy Sci 95: 7051–7065

Patel VR, Kansara JD, Patel BB, Patel PB, Patel SB (2011) Prevention of Milk fever: Nutritional Approach. Vet World 4**:** 278–280

Ramberg CF, Mayer GP, Kronfeld DS, Phang JM, Berman M (1984) Calcium homeostasis in cows, with special reference to parturient hypocalcaemia. Am J Physiol 246:698–704

Razzaghi A. Aliarabi H. Tabatabaei MM, Saki AA, Valizadeh R, Zamani P (2012) Effect of Dietary Cation-Anion Difference during Prepartum and Postpartum Periods on Performance, Blood and Urine Minerals Status of Holstein Dairy Cow. Asian-Australas J Anim Sci 25: 486–495

Reinhardt TA, Lippolis JD, Mccluskey BJ, Goff JP, Horst RL (2011) Prevalence of subclinical hypocalcemia in dairy herds. Vet J 188: 122–124

Rossato W, Gonzalez FH, Dias MM, Riccó D, Valle SF, Rosa VL, Conceição T, Duarte F, Wald V. (2001) Number of lactations affects metabolic profile of dairy cows. Archive Vet Sci 6:83–88

Schonewille JT, Van't Klooster AT, Dirkswager A, Bayen A (1994) Stimulatory effect of an anion (chloride)-rich ration on apparent calcium absorption in dairy cows. Livestock Prod Sci 40: 233–24

Seifi HA, Mohri M, Ehsani A, Hosseini E, Chamsaz M (2005) Interpretation of bovine serum total calcium: Effects of adjustment for albumin and total protein. Comp Clin Pathol 14: 155-159

Shahzad AM, Sarwar M, Mahr-un-Nisa (2008) Influence of varying dietary cation anion difference on serum minerals, mineral balance and hypocalcemia in Nili Ravi buffaloes. Livestock Sci 113**:** 52–61

Shahzad M, Sharif M, Nisa M, Sarwar M, Khalid MF, Saddiqi HA (2011) Changing certain dietary cationic and anionic minerals: Impact on blood chemistry, milk fever and udder edema in buffaloes during winter. African J Biotech 10: 13651-13663

Singh G (2014) Metabolic profiling, therapeutic and prophylactic studies on metabolic diseases in crossbred cows from Punjab [PhD]: Guru Angad Dev Vet. and Animal Sciences University.

Tucker WB, Xin Z, Hemken R W (1988b) Influence of dietary calcium chloride on adaptive changes in acid-base status and mineral metabolism in lactating dairy cows fed a diet high in sodium bicarbonate. J Dairy Sci 71**:** 1587–1597

Wu WX, Liu J X, Xu G Z, Ye JA (2008) Calcium homeostasis, acid-base balance, and health status in periparturient Holstein cows fed diets with low cation-anion difference. *Livestock Sci* 117: 7–14

Yatoo M, Parray O, Ashraf T, Ashraf S, Nisar M, Taifah S (2018) Longitudinal study on evaluation of serum micromineral and hormone interrelation in cattle. J Pharmaco Phytochem 7: 1201–1203