

CORRELATION OF SERUM CALCIUM WITH ENZYMES ASPARTATE AMINOTRANSFERASE, CREATINE KINASE AND MASTITIS IN POSTPARTUM PERIOD IN DAIRY CATTLE

UMAR ARSHAD¹, QAMAR-UN-NISA^{1*}, WAQAS AHMAD², MUBASHER RAUF³

¹Department of Pathology, University of Veterinary and Animal Sciences, Lahore-Pakistan

²Department of epidemiology, KBCMA, Narowal University of Veterinary and Animal Sciences, Lahore-Pakistan

³Department of Pathology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur-Pakistan

ARTICLE INFORMATION

Article History:

Received: 07th December 2022

Accepted: 21st June 2023

Published online: 30th June 2023

Author's contribution

UA designed and conducted research plan, QN reviewed the article, WA wrote manuscript, MR applied statistics

Key words:

Mastitis , Dairy cattle , AST, SCC and CK

ABSTRACT

Mastitis is inflammation of udder and its chances increases in hypocalcemia cases because sphincters of teat are loosened due to calcium-ion channel blockage. Cows those suffered milk fever have 8 times more chances of developing mastitis than other cows which haven't suffered hypocalcaemia. The values of AST and CK are high in downer cows due to hypocalcemia as compared to healthy cows. Subclinical hypocalcaemia is one of the major complication occurring just after parturition in local and exotic breeds of cattle in Pakistan. It weakens the immune system of dairy animal and also decreases the production potential of animals thus causing serious economic losses in terms of decrease milk production of animal, decrease earning of animal and increased treatment cost due to secondary infection like mastitis occurred due to weakened immune function. The current study was aimed to the correlation of serum calcium with enzymes Creatine Kinase and Aspartate amino transferase and mastitis in postpartum period in dairy cattle. A total of 50 cattle (n=50) just after parturition were included in this study. The cattle were sampled in two ways. Firstly, 10ml blood was drawn aseptically into clot activator vacutainers from jugular vein. Secondly milk samples were taken from all four teats. First 2 milk strips were discarded and next strips were collected in aseptic test tubes. The samples were transported to Pathology laboratory, Department of Pathology, University of Veterinary and Animal Sciences, Lahore maintaining the cold chain and stored at -20 °C till further analysis. Correlation between calcium and creatin kinase comes out to be 0.279 which is weak positive correlation. Correlation between calcium and aspartate aminotransferase is also weak and comes out to be 0.111. Similarly, correlation between calcium and SCC also weak and comes out to be 0.193. This is the first study of correlation of calcium with CK, AST and SCC in dairy cattle after postpartum period.

1. INTRODUCTION

Calcium is a macro-mineral that plays an important function in the body which are bone matrix formation, process of muscle contraction and transmission of nerve impulses. Ionized calcium is also important for essential cellular functions like signaling, neurotransmission, muscle contraction, metabolism, growth and proliferation and triggering of immune responses (Saris & Carafoli 2005), (Vig & Kinet 2009).

Normal blood calcium in the adult cows is maintained between 8.5 and 10 mg/dl (Goff 2008). Maintenance of blood calcium with acceptable is a balancing act between calcium demand of milk production and the cow's homeostatic mechanism to maintain blood calcium. During the last trimester in pregnancy supply of calcium through diet is more than adequate to maintain homeostasis without the activating calcium mobilization system which is activated after parturition (Taylor et al. 2001)

*Corresponding Author: qamar.nisa@uvas.edu.pk

Copyright 2017 University of Sindh Journal of Animal Sciences

Hypocalcemia is one of the most common mineral related metabolic disorders of milking animals which occurs at low level of blood calcium. It happens immediately after parturition or just before parturition. A mild degree of hypocalcaemia also leads to conditions like uterine prolapse, retained placenta, metritis, mastitis, ruminal stasis, decrease of immune response and reproductive performance (Khan et al. 2015).

AST activity is widely studied in both animals and humans. It is found that variation of AST level had relations with the liver functioning, skeletal muscle performance and heart muscles of cows (Kaneko et al. 2008). The values of AST and CK are high in downer cows as compared to healthy ones. Ischaemic necrosis results in higher values of AST and CK in circulation due to prolonged recumbancy (Cox et al. 1982). In these factors one is calcium level, if its value drops from normal level in blood or serum then calcium-ion channels providing help in muscle contraction are affected and results in muscle damage. As muscle cell degradation occurs in forced calving or dystocia cases and if level of calcium is also low from normal value it will result in muscle damage and level of CK will be high in serum. Hypocalcemia patient without signs of heart muscle damage and other dystrophies show elevated signs of serum creatinine kinase and high chances of elevation are there in cases of severe hypocalcemia.

Subclinical hypocalcaemia is one of the major complications occurring just after parturition in local and exotic breeds of cattle in Pakistan. It weakens the immune system of dairy animal and also decrease the production potential of animals thus causing serious economic losses in terms of decrease milk production of animal, decrease earning of animal and increased treatment cost due to secondary infection like mastitis occurred due to weakened immune function. Keeping in view the importance of this health malady of cattle and its serious economic concerns the current study has been designed.

2. MATERIALS AND METHODS

Study area and Study animal

The study was carried out in district Sheikhpura at various private and corporate dairy farms and small households. A total of 50 cattle (n=50) just after parturition were included in this study.

Inclusion Criteria was those cattle just after parturition or showing any of following clinical signs like inflammation of udder, anorexia, depression, weakness and subnormal temperature were included in this study.

Digestion of blood sample for calcium analysis

1ml sample was taken for the analysis. 7ml HNO₃ (nitric acid) was added. Then 3ml of HClO₄ (per chloric acid) was added. Sample was then kept in digestion block at 150°C for 30 minutes. After this temperature was raised to 250°C and it was kept for 15 minutes till point is achieved i.e wine-green/crystal clear. Sample was then cooled and final volume 25ml with distilled water was made. Calcium analysis on Flame Photometer Standardize against standard. 1ppm, 5ppm, 10ppm, 15ppm, 20ppm, 25ppm, 30ppm, 35ppm, 40ppm.

Sampling procedure

The cattle were sampled in two ways. Firstly, 10ml blood was drawn aseptically into clot activator vacutainers from jugular vein. The samples were transported to the Pathology laboratory in Department of Pathology, University of Veterinary and Animal Sciences, Lahore maintaining the cold chain. The serum in clot activator vacutainers was stored at -20°C till the further analyses. The data regarding owner, animal, management and environmental factors was recorded on data capture form for each sampling unit.

Secondly milk samples were taken from all four teats First 2 milk strips were discarded and next strips were collected in aseptic test tubes. The samples were transported to Pathology laboratory in Department of Pathology, University of Veterinary and Animal Sciences, Lahore maintaining the cold chain.

Serum analysis

Serum was analyzed for Aspartate aminotransferase (AST) and creatinine phosphokinase (CPK) using semiautomatic biochemical serum analyzer following manufacturer's direction.

Milk analysis

Milk was analyzed for somatic cell count (SCC) through California Mastitis Kit (CMT). Milk sample were collected from all 4 quarters in a clean CMT paddle which has 4 cups marked A, B, C, D for all four quarters. Take a tea spoon of milk (2cc) in 4 cups from four quarters. Equal amount of CMT solution was then added in each cup of paddle. CMT paddle was rotated in circular motion to mix

thoroughly. Sample was not mixed for more than 10 seconds. Visible reaction disintegrates after 20 seconds. Reaction is stored visually. More the gel formation, higher is the score.

3. RESULTS AND DISCUSSION

The mean of calcium in 50 dairy cattle suffering from sub clinical hypocalcemia was 7.042mg/dl in current study with standard deviation of 0.88158 while the mean of creatine kinase (CK) in these animals was 243.02 U/L with standard deviation of 55.356

Calcium and CK has statistically non-significant relationship ($p>0.05$).

The direction of relationship is positive (i.e calcium and CK are positively correlated) meanings that these variables tends to increase together.

The strength of correlation between two variables is approximately weak.

The mean of calcium in 50 dairy cattle suffering from sub clinical hypocalcemia was 7.042mg/dl in current study with standard deviation of 0.88158 while the mean of aspartate aminotransferase (AST) in these animals was 183.30 U/L with standard deviation of 34.195.

Calcium and AST has statistically non-significant relationship ($p>0.05$).

The direction of relationship is positive (i.e calcium and AST are positively correlated) meanings that these variables tends to increase together.

The strength of correlation between two variables is very weak.

The mean of calcium in 50 dairy cattle suffering from sub clinical hypocalcemia was 7.042mg/dl in current study with standard deviation of 0.88158 while the mean of somatic cell count in these animals was 274426 with standard deviation of 400012.52981.

Calcium and Somatic cell count has statistically non-significant relationship ($p>0.05$).

The direction of relationship is positive (i.e calcium and AST are positively correlated) meanings that these variables tends to increase together.

The strength of correlation between two variables is very weak.

Livestock plays an important role for the uplift of rural economy by contributing 11.8% in national GDP and 55.5% in the agriculture sector's share of GDP. It is considered a source of employment generation in rural

areas, helping reduce variation in income. It is central to the livelihood of the rural poor and can play an important role in poverty alleviation.

Milk fever or subclinical hypocalcemia is a very fatal disease in dairy animals which is caused by decrease of calcium level in the body of dairy animals immediately after parturition or just before parturition. About 50% or more animals in second or third lactation are more prone to milk fever due to increase of milk production in second or third lactation. It causes severe economic losses to farmers in terms of decrease milk production and medicate on costs. It also causes production to decrease throughout the lactation period. (Fikadu et al. 2016).

The Government believes that increase in livestock production can have a strong impact on the incomes of the rural poor. Productivity increase in livestock and dairy can have significant positive impact on total income in the sector as well as exports. There is substantial scope in increasing the export of high-value livestock and dairy value-added products. At this time, some of the best strategies for us to avoid losses due to infectious disease are to pay strict attention to the details of close-up and fresh cow management such that metabolic insults to the immune system are avoided. Further research elucidating endocrine, metabolic, and immune interactions around the time of calving are warranted. In addition to metabolites, many dietary nutrients are involved in immune protection and may play roles in immunosuppression.

Dairy cows under stress condition use fat reserves which results in cell structure change and damage to cell structure and disturbance in values of AST and CK. (Rukkamsuk et al. 2000). Decrease in calcium level in the body effects certain functions in the body metabolism and functioning like liver lipid infiltration, effect on enzymes values in serum, deformities and osteoporosis in bones and muscles contraction and relaxation are also highly effected from this.

Moreover, cows effected with milk fever are more prone approximately 9% more to ketosis then those who are not effected with milk fever. The samples are processed for checking the levels of enzymes and calcium to check the effect of decrease calcium on body cells and functioning. The skeleton of a 600-kg cow contains approximately 8.5 kg of Ca. There are 3 g Ca in the plasma pool and only 8 to 9 g Ca in all the extracellular fluids of a 600-kg cow.

Blood Ca in the adult cow is maintained between 2.1 and 2.5 mmol/L (8.5 and 10 mg/dL) and is slightly higher in young animals. About 50% of the blood Ca is bound to proteins such as albumin, less than 10% is in mineral complexes with inorganic phosphates and the remainder exists in the ionized form. The ionized Ca concentration is the biologically active form of the Ca in blood and is most important for immediate metabolic function. (Breves 1995).

As calcium is a macro mineral which also plays a role in transmission of nerve impulses due to which a decrease in its level causes effects on neurotransmission, muscle functioning and immune cell activation. In the current study 50 samples were taken from cattles in different house holding areas in district Sheikhpura and samples were taken from those cattles which were freshly parturated and these samples were taken in clot activator vacutainers and were transported to laboratory by maintaining proper cold chain.

The study done is to find out the relation between calcium and enzymes. Milk samples were also taken from the same cows and were sent to the laboratory by maintaining cold chain for the somatic cell count and serum was extracted from blood samples for checking the levels of AST and CK. (Kimura et al. 2006).

The correlation in the current studies is weak mostly similar to the previous researches but a bit high as compared to the researches in foreign which clearly indicates managerial issues in feeding and cleaning for enzymes and mastitis respectively. This study finding are quiet similar to the findings of the researches done before and have a little bit variation.

The outcome of study done yield data about the status of sub-clinical hypocalcemia in cattle population of district Sheikhpura and its effect on serum enzymes like AST and CPK and its relationship with mastitis. The study findings helped in suggesting some strict measures for minimizing economic losses in dairy industry of Pakistan.

4. CONCLUSION

The study reveals that the requirement of calcium in the body should be maintained by a proper diet containing an adequate amount of calcium to fulfill the body's needs and ensure the proper functioning of enzymes and muscles. If

the diet is low in calcium, it should be supplemented with external calcium, such as Dicalcium phosphate in the diet. Proper management of affected animals through treatment is also essential.

5. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

REFERENCES

- Breves G (1995). Gastrointestinal calcium and phosphate metabolism in ruminants. In *Ruminant Physiology: Digestion and Metabolism, Growth and Reproduction: Proceeding of the 8th International Symposium on Rumina* pp. 135-151. Academic Press.
- Cox, V. S., McGrath, C. J., & Jorgensen, S. E. (1982). The role of pressure damage in pathogenesis of the downer cow syndrome. *American Journal of Veterinary Research*, 43(1), 26-31.
- Fikadu, W., Tegegne, D., Abdela, N., & Ahmed, W. M. (2016). Milk fever and its economic consequences in dairy cows: a review. *Global veterinaria*, 16(5), 441-452.
- Goff, J. P. (2008). The monitoring, prevention, and treatment of milk fever and subclinical hypocalcemia in dairy cows. *The veterinary journal*, 176(1), 50-57.
- Kaneko, J. J., Harvey, J. W., & Bruss, M. L. (Eds.). (2008). *Clinical biochemistry of domestic animals*. Academic press.
- Khan, A., Mushtaq, M. H., Khan, A. W., Chaudhry, M., & Hussain, A. (2015). Descriptive epidemiology and seasonal variation in prevalence of milk fever in KPK (Pakistan). *Global Veterinarian*, 14(4), 472-477.
- Kimura K, Reinhardt T, Goff J. 2006. Parturition and Hypocalcemia Blunts Calcium Signals in Immune Cells of Dairy Cattle. *J Dairy Sci*. 89 (7): 2588-2595.
- Rukkwamsuk, T., Geelen, M. J. H., Kruij, T. A. M., & Wensing, T. (2000). Interrelation of fatty acid composition in adipose tissue, serum, and liver of dairy cows during the development of fatty liver postpartum. *Journal of dairy science*, 83(1), 52-59.
- Saris, N. E. L., & Carafoli, E. (2005). A historical review of cellular calcium handling, with emphasis on mitochondria. *Biochemistry (Moscow)*, 70(2), 187-194.
- Taylor, V. J., Hattan, A. J., Bleach, E. C., Beever, D. E., & Wathes, D. C. (2001). Reproductive function

in average and high yielding dairy cows. *BSAP Occasional Publication*, 26(2), 495-498.

Vig, M., & Kinet, J. P. (2009). Calcium signaling in immune cells. *Nature immunology*, 10(1), 21-27.



Figure 1. Map of District Sheikhupura

Table 1. Summary Statistics of Calcium and CK Level

	Mean	Std. Deviation	N
Calcium	7.0420	.88158	50
CK	243.02	55.356	50

Table 2. Correlation of Calcium with CK

		Calcium	CK
Calcium	Pearson Correlation	1	0.279
	p-value		0.050
	N	50	50
CK	Pearson Correlation	0.279	1
	p-value	0.050	
	N	50	50

Table 3. Summary Statistics of Calcium and SCC Levels

	Mean	Std. Deviation	N
Calcium	7.0420	0.88158	50
SCC	274426.000	400012.52981	50

Table 4. Summary Statistics of Calcium and AST Levels

	Mean	Std. Deviation	N
Calcium	7.0420	0.88158	50
AST	183.30	34.195	50

Table 5. Correlation of Calcium with AST

		Calcium	AST
Calcium	Pearson Correlation	1	0.111
	p-value		0.442
	N	50	50
AST	Pearson Correlation	0.111	1
	p-value	0.442	
	N	50	50

Table 6. Correlation of Calcium with Mastitis (Somatic Cell Count)

		Calcium	SCC
Calcium	Pearson Correlation	1	.193
	p-value		.178
	N	50	50
SCC	Pearson Correlation	.193	1
	p-value	.178	
	N	50	50

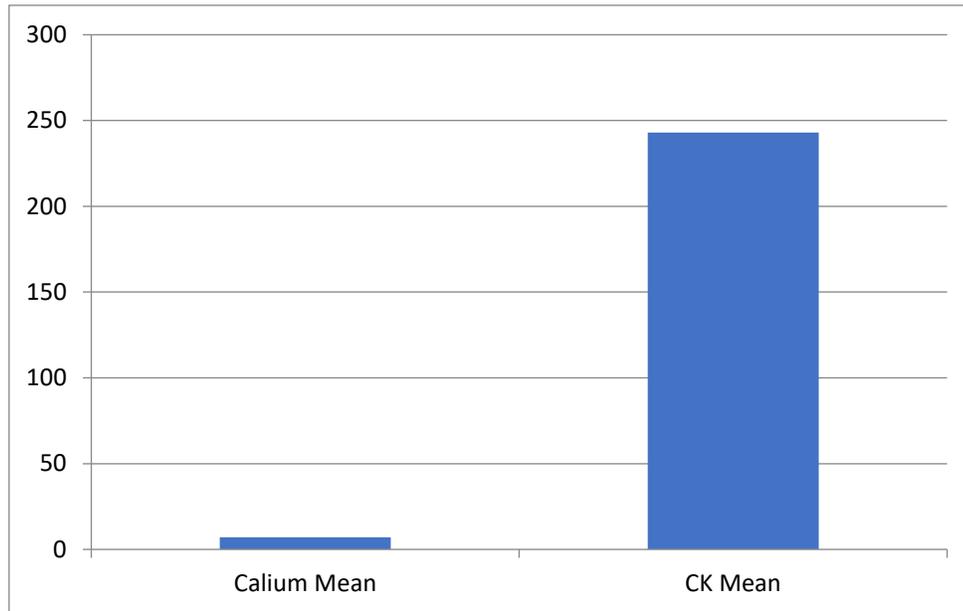


Figure 2. Comparison of mean of calcium and creatine kinase (CK) in infected animals.

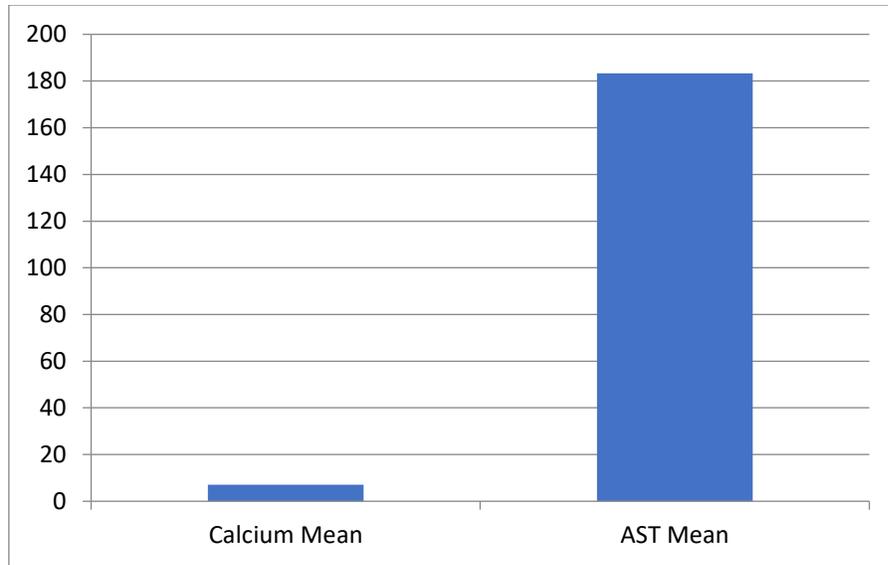


Figure 3. Comparison of mean of calcium and aspartate aminotransferase (AST) in infected animals

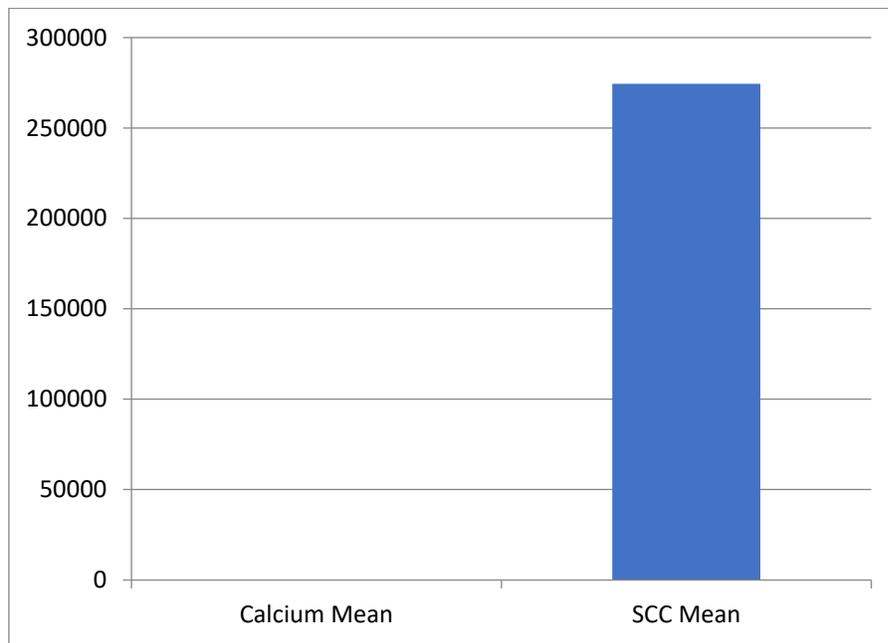


Figure 4. Comparison of mean of calcium and somatic cell count (SCC) in infected animals