Original Research

Reproductive Disorders and Its Relationship with Hormones and Mineral Status in Bovines of Organized Dairy Farms

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Abstract

In the present study, relationship of reproductive disorders with mineral and hormone status in bovines was analyzed. Paired sera samples (160) were collected randomly from four organized dairy farms in Karnataka (Bijapur, Dharwad) and Tamilnadu (Chennai, Cuddalore). Serum estradiol, progesterone in healthy and RP animals were 11.33±0.58 and 5.67±0.10pg/ml, 1.70±0.08 and 0.77±0.04IU/ml, respectively and significant (P<0.05) decrease in RP bovines. Serum copper, zinc, calcium, magnesium, phosphorus in healthy and RP bovines were 1.29±0.03 and 0.91±0.03ppm, 1.09±0.04 and 0.87±0.03ppm, 9.98±0.17 and 9.07±0.21mg/dl, 3.07±0.16 and 2.36±0.08mg/dl, 1.62±0.08 and 1.52±0.06mg/dl, respectively. Significant (P<0.05) correlation between copper and hormones in bovines of organized dairy farms was observed. Logit analysis revealed significant (P<0.05) positive relationship between estradiol, progesterone and zinc levels with animal health status. Thus, supplementation of these microminerals will reduce the incidence of reproductive disorders in animals and also improves the productivity and profitability of dairy farmers in India.

Key words: Reproductive Problems, Hormones, Minerals, Correlation, Logit analysis

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Introduction

The reproductive problems are the main impediments for the profitable dairying like abortions, repeat breeding, retained placenta, anoestrus, etc. Mineral deficiencies, imbalances and toxicity of certain

mineral elements may cause reproductive disorders as minerals play an important role in health and reproduction of the livestock (Sharma *et al.*, 2007). Minerals are required for normal functioning of basically all biochemical processes of the body and acts as catalysts in both enzyme and hormones systems (Suttle, 2010). After energy and protein, minerals are the major nutrients required and should be given priority in order to optimize reproduction in dairy cattle (Bindari *et al*., 2013). Hormones and minerals are required at optimum levels to avoid reproductive problems (RP) in bovines of organised dairy farms. Besides energy and protein, deficiency of these elements such as calcium, phosphorus, iron, zinc, copper, etc., in blood have been reported to be a predisposing factor for the occurrence of retention of placenta and repeat breeding in dairy cows (Sheetal *et al.*, 2014; Kumar, 2014).

Metallo-enzymes, of which essential minerals are constituents, are important in the synthesis of many steroid hormones (Yamaguchi *et al*., 2009). The occurrence of reproductive problems in dairy animals is dependent on various factors like nutrient intake, physiological conditions, management and climatic conditions, etc. The mineral requirement of bovines depends on the age, species, breed, physiological conditions and reproductive status of animals. The animals get minerals through the consumption of natural feeds, fodders and supplementation of inorganic salts in the ration; however it may be sufficient or insufficient and depends on the digestion, absorption and other factors. It is necessary to provide required minerals in the feed for the better productivity of animals in organized dairy farms. Optimum level of zinc was essential to maintain the activity of follicle stimulating hormone and luteinizing hormone and thereby facilitate the normal reproductive performance (Kapadiya and Siddiquee, 2012). Mineral deficiency affects hormone status and impairs the production potential of animals (Yatoo *et al*., 2013). Minerals and mineral-hormone interactions in bovines have not been studied especially in developing countries where the mineral deficiencies are very common. There was limited literature on the study of relationship of minerals and hormones with reproductive disorders in diary animals of organized dairy farms in India. Hence, the present study was undertaken to investigate the relationship of hormones and minerals levels in dairy animals with their reproductive problems.

Materials and Methods

Selection of Farm and Animals

Four organised dairy farms located in Bijapur, Dharwad in Karnataka (Farm A, B) and Chennai, Cuddalore in Tamilnadu (Farm C, D) in southern India were selected randomly for this study. The history of the animals like sex, species, breed, age, animal health status and managemental conditions of the organised dairy farms were collected. Based on the history of the animals, twenty paired serum samples (one month interval) each from apparently healthy animals and reproductive problem (RP) animals from each farm were collected. A total of 160 serum samples, from cattle (129) and buffaloes (31), were

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collected from four dairy farms for the study. The blood samples were collected by using serum vacutainer tubes coated with silicone and micronized silica particles to accelerate clotting and multi sample vacutainer needles (Becton and Dickenson Company, USA). The serum was separated by centrifugation at 2000 rpm for 20 minutes. All serum samples were stored at -20° C until used for estimation. Samples from female animals were collected for this study. Based on breeds of cattle, Jersey cross (58), Holstein Friesian cross (47), Sahiwal (9), Deoni (5), Rathi (5), Tharparkar (5) and among buffalo breeds, Murrah (31) were tested. The animals were classified based on age, by the number of lactations as 1 calving (35), 2 calvings (55), 3 calvings (48), 4 calvings (14) and 5 calvings (8). Based on animal health status, the animals were categorised as apparently healthy (63), pregnant (17), repeat breeding (58), abortions (13), metritis (5) and mastitis (4).

Hormone and Mineral Estimation

The bovines were grouped in two groups based on animal health status as apparently healthy and reproductive problem animals for each farms. Twenty sera samples (n=20) were from each of the two categories of animals, in each farm and used for estimation of hormones. Serum estrogen (E_2), progesterone (P_4) was determined by radioimmunoassay (RIA) technique using Gamma Scintillation counter (I^{125} calibrated), at Animal Physiology Division, ICAR-National Institute of Animal Nutrition and Physiology (NIANP), Bengaluru. Estradiol and Progesterone radioimmunoassay kit was obtained from Immunotech, France and carried as per the manufacturer's instructions. The paired serum samples were used and average of the two samples per animal was considered for analysis purpose. A total of 160 serum samples from the category of apparently healthy (80) and reproductive problem animals (80) were used for estimation of serum minerals. The serum samples were suitably aliquoted and wet digested with nitric acid and perchloric acid mixture (3:1) for preparing the acid mineral extract. Calcium (Ca), magnesium (Mg), copper (Cu) and zinc (Zn) were estimated as per the method reported earlier (Oser, 1979) using atomic absorption spectrophotometer (Perkin Elmer AA 300). For estimation of Ca and Mg, acid extracts were suitably diluted (1:50 or 1:100) with 0.1% lanthanum chloride to avoid interference from phosphates. Phosphorus was estimated colorimetrically (AOAC, 1990). Mineral standards were run for each analysis and minerals.

Statistical Analysis

The data analysis by calculating descriptive statistics, one way analysis of variance and correlation coefficient as described method earlier (Snedecor and Cochran, 1989) and using Statistical Analysis System (SAS) Software Enterprise Guide version 5.1 (SAS India Ltd, Mumbai, India). The logit analysis was carried out by using SPSS (Statistical Package for Social Sciences) software version 22.0 (IBM India

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Private Limited, Bengaluru). Logit model for discrete dependent variables taking binary value of either 0 or 1 was used for the analysis. The dependent variable takes the value 1 with a probability of success (p), or the value) with probability of failure (1-p). The dependent variable in the Logit regression analysis was dichotomous with 1 (healthy) and 0 (reproductive problem animals) in this study. This type of variable is called a Bernoulli (or binary) variable. Logistic regression makes no assumption about the distribution of the independent variables. They do have to be normally distributed, linearly related or of equal variance within each group. The relationship between the predictor and response variables is not a linear function in logistic regression; instead, the logistic regression function is used, which is the logit transformation of *p*.

$$P_i = \frac{e^{(\beta 0 + \beta 1 X_1 + \ldots\ldots\ldots\ldots + \beta n X_n + \mu_i)}}{1 + e^{(\beta 0 + \beta 1 X_1 + \ldots\ldots\ldots\ldots + \beta n X_n + \mu_i)}}$$

Where,

Pi = (1 for healthy and 0 for reproductive problem animals);

β0.....βn are the parameters to be estimated or the coefficient of the predictor variables;

X_1..... X_n is the number of independent or predictor variables used in the model;

"i" is the ith observation; and μi is the random error.

Since logistic regression calculates the probability of success (p) over the probability of failure (q), the results of the analysis are in the form of an odds ratio (p/q). The best fit Logit model was adjudged based upon the likelihood ratio which is denoted as -2 log likelihood (-2LL). The minimum value of -2 log likelihood is 0, which corresponds to a perfect fit, hence; the lower its value the better is the model. Nagelkerke R2 value provides the basis to represent the overall model fit. Wald statistics provides the statistical significance for each estimated coefficient (β). The estimated coefficients (β0 ..... βn) represent the influence of a variable on the dependent variable. Since the model is in non-linear form, it is difficult to interpret the coefficients directly. For better interpretation the coefficients are transformed into Odds' ratio (Hair *et al*., 2007).

Results

The Mean ± SE of serum hormone and mineral contents of bovines in organized dairy farms are given in Table 1. The mean ±SE serum estradiol, progesterone concentration in healthy and RP animals were 11.33±0.58 and 5.67±0.10 pg/ml, 1.70±0.08 and 0.77±0.04 IU/ml, respectively. The hormones, serum estradiol and progesterone levels showed the significant (P<0.05) decrease in animals with reproductive problems when compared to healthy animals. The mean ±SE serum copper, zinc, calcium, magnesium,

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phosphorus in healthy and RP bovines were 1.29±0.03 and 0.91±0.03 ppm, 1.09±0.04 and 0.87±0.03 ppm, 9.98±0.17 and 9.07±0.21 mg/dl, 3.07±0.16 and 2.36±0.08 mg/dl, 1.62±0.08 and 1.52±0.06 mg/dl, respectively.

Table 1: Serum hormone and mineral levels of bovines in four organized dairy farms

No.	Dairy Farms Location	Animal Status (n=20)	Estradiol (pg/ml)	Progesterone (IU/ml)	Copper (ppm)	Zinc (ppm)	Calcium (mg/dl)	Phosphorus (mg/dl)	Magnesium (mg/dl)
1	Farm A Bijapur	Apparently Healthy	11.71 ± 2.09*	1.89 ± 0.27*	1.57 ± 0.07*	1.40 ± 0.08*	10.72 ± 0.43	2.76 ± 0.27	2.17 ± 0.26
		Reproductive problems	4.94 ± 0.11*	0.92 ± 0.08*	0.98 ± 0.09*	1.07 ± 0.03*	10.27 ± 0.27	2.21 ± 0.15	1.71 ± 0.08
2	Farm B Dharwad	Apparently Healthy	12.53 ± 0.50*	1.85 ± 0.13*	1.27 ± 0.06*	1.04 ± 0.08	9.23 ± 0.40*	2.47 ± 0.20	1.22 ± 0.06*
		Reproductive problems	5.91 ± 0.15*	0.70 ± 0.07*	0.86 ± 0.03*	0.90 ± 0.05	6.65 ± 0.33*	2.19 ± 0.14	0.98 ± 0.06*
3	Farm C Chennai	Apparently Healthy	10.83 ± 0.84*	1.56 ± 0.05*	1.13 ± 0.04*	1.02 ± 0.05*	10.11 ± 0.25	4.62 ± 0.31*	1.70 ± 0.08
		Reproductive problems	5.95 ± 0.23*	0.89 ± 0.09*	0.89 ± 0.03*	0.84 ± 0.05*	9.91 ± 0.26	2.88 ± 0.17*	1.56 ± 0.04
4	Farm D Cuddalore	Apparently Healthy	10.24 ± 0.34*	1.49 ± 0.06*	1.16 ± 0.04*	0.88 ± 0.03*	9.84 ± 0.16	2.40 ± 0.14	1.39±0.09[b]*
		Reproductive problems	5.89 ± 0.20*	0.59 ± 0.05*	0.90 ± 0.03*	0.68 ± 0.05*	9.45 ± 0.14	2.18 ± 0.14	1.83 ± 0.14*
5	Total	Apparently Healthy (n=80)	11.33 ± 0.58*	1.70 ± 0.08*	1.29 ± 0.03*	1.09 ± 0.04*	9.98 ± 0.17*	3.07 ± 0.16*	1.62 ± 0.08
		Reproductive problems (n==80)	5.67 ± 0.10*	0.77 ± 0.04*	0.91 ± 0.03*	0.87 ± 0.03*	9.07 ± 0.21*	2.36 ± 0.08*	1.52 ± 0.06

Apparently healthy- Animals which was looking normal and without any clinical signs; Reproductive problems- Animals with history of abortion, repeat breeding, metritis and mastitis

** Significant (P<0.05) difference between the apparently healthy and reproductive problems group within the farms*

The serum copper showed significant (P<0.05) decrease in reproductive problem animals in all the four farms studied. Zinc concentration in serum revealed significant (P<0.05) decrease in three farms but not in Dharwad farm. The serum calcium levels revealed no significant difference between the healthy and reproductive problem animals except Dharwad farm. Phosphorus levels showed no significant difference but farm in Chennai had significant difference between the two categories of animals. The magnesium concentration showed no significant difference between the apparently healthy and reproductive problem animals in Bijapur and Chennai farms but significant (P<0.05) difference in Dharwad and Cuddalore farms were observed. Overall, there was decrease in the levels of hormones and minerals in animals with reproductive problems when compared to apparently healthy animals were observed indicating their importance in reproduction of bovines.

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The correlation coefficient between the serum hormones and minerals is given in Table 2. The significant (P<0.05) correlation was observed between the copper with estradiol and progesterone hormones. Magnesium showed significant (P<0.05) correlation with estradiol hormone.

Table 2: Correlation coefficient between minerals and hormones levels in bovines

Minerals	Estradiol (E_2)	Progesterone (P_4)
Copper (Cu)	0.48*	0.50*
Zinc (Zn)	0.18	0.21
Calcium (Ca)	0.23	0.21
Phosphorus (P)	0.22	0.16
Magnesium (Mg)	0.32*	0.04

** Significant correlation (P<0.05)*

Minerals like zinc, calcium, phosphorus do not have correlation with estradiol and progesterone hormones. The definition and measurement of various variables used of Logit analysis is given in Table 3.

Table 3: Definition of variables and its measurement used in Logit analysis

Variable	Definition and Measurement
Animal Health Status (Y)	Animals with history of reproductive problems-0, Animals which was apparently healthy, without clinical signs and no history of reproductive problems-1
Age Category (X_1)	Age of the cattle and buffalo, measured in number of lactations; equal to and above four calvings-0, equal to and below three calvings-1
Breed Category (X_2)	Breed of animals are described based on certain genetic characteristic features; Crossbreds-0, Indigenous breeds-1
Estradiol (X_3)	Hormone secreted during oestrus cycle of animals; measured in picogram per mililitre in serum
Progesterone (X_4)	Progesterone hormone secreted during oestrus cycle and during pregnancy; measured in International units per millilitre in serum
Copper (X_5)	Copper is a trace mineral; measured in parts per million in serum
Zinc (X_6)	Zinc is a trace mineral; measured in parts per million in serum
Calcium (X_7)	Calcium is a macro mineral of the body; measured in milligrams per decilitre in serum
Phosphorus (X_8)	Phosphorus is a macro mineral present in the cells; measured in milligrams per decilitre in serum
Magnesium (X_9)	Magnesium is macro mineral in the body; measured in milligrams per decilitre in serum

The regression analysis results of Logit model is given in Table 4, which showed the coefficients (B), their standard errors, the Wald Chi-square statistic, associated p-values and odds ratio (Exp (B)). The significant chi-square value and Nagerkerke R2 value (0.963) revealed that the overall fit of the model

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was better. The positive values of significant coefficients of explanatory variables indicated their positive influences on health status of animals in organized dairy farms. There was significant (P<0.05) influence of progesterone and zinc levels in determining the health status of bovines. Estradiol levels showed highly significant (P<0.01) relationship in determining the animal health status of bovines in organized dairy farms.

Table 4: Regression analysis result of Logit regression model for health status in bovines

Variable	B	Standard Error	Wald	Degrees of Freedom	Significance P-value	Exp (B)
Constant	-31.160	8.374	13.846	1	0.000	0.000
Age category	0.649	2.558	0.064	1	0.800	1.914
Breed category	1.961	1.444	1.843	1	0.175	7.105
Estradiol	1.588	0.487	10.643	1	0.001**	4.893
Progesterone	4.317	1.716	6.327	1	0.012*	74.951
Copper	2.587	2.058	1.580	1	0.209	13.288
Zinc	6.284	2.822	4.960	1	0.026*	536.047
Calcium	0.266	0.403	0.436	1	0.509	1.305
Phosphorus	0.740	0.547	1.830	1	0.176	2.097
Magnesium	0.062	1.216	0.003	1	0.959	1.064

*Chi square = 3.037 (P<0.01); -2 log likelihood = 25.403; Nagelkerke R square = 0.943; Accuracy of prediction of classes = 96.3%; Level of significance: ** (P<0.01); *(P<0.05)*

Discussion

The hormones, serum estradiol and progesterone levels showed the significant (P<0.05) decrease in animals with reproductive problems compared to healthy animals. This might be due to the fact that the animals were suffering from various reproductive problems due to decreased hormone levels and depends on various factors like nutrition, age, management, etc. The serum hormone values observed in the present study concurred with previous report (Yatoo *et al*., 2013). The serum minerals like copper, zinc, calcium and phosphorus showed significant decrease in the bovines with reproductive problems than the healthy counterparts. However, the magnesium revealed a non significant decrease between the healthy and reproductive problem bovines. In a study on trace elements in repeat breeding Mehsana buffaloes showed significant decrease in copper and zinc levels compared to healthy buffaloes (Kapadiya and Siddiquee, 2012). The copper and zinc values in blood serum were significantly higher in normal than repeat breeder Kankrej cows (Modi *et al*., 2013). The results in the present study observed for serum copper and zinc levels, concurred with previous studies (Kapadiya and Siddiquee; 2012, Modi *et al*., 2013). Zinc deficiency results in alteration of steroidogenesis (Yamaguchi *et al*., 2009). Calcium plays an important role in various physiological functions and lactating cows should be provided with adequate calcium in feed to maximize production and minimize health problems. Calcium deficiency causes

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reduced muscle tone in uterus leading to prolonged calving, retained placenta and giving rise to fertility problems in cows. It also causes suppression of dry matter intake, increase in body fat mobilization in the form of non-esterified fatty acids and reduction of neutrophil function (Martinez *et al*., 2014). Phosphorus deficiency leads to decline in fertility rate, feed intake, milk production, ovarian activity, irregular oestrous cycle, increased occurrence of cystic ovaries, delayed sexual maturity and low conception rates (Cromwell, 1997) and indicated the importance of these minerals in health status of dairy animals. Magnesium usually does not have direct impact on the reproductive status of animals, since in body it remains in almost antagonistic relation with calcium and any disturbance in Ca-P-Mg homeostasis can impart some influence on reproduction. Moreover, magnesium deficiency causes loss of appetite which in turn reduces reproductive efficiency in bovines (Sathish Kumar, 2003).

Minerals like zinc, calcium, phosphorus revealed no correlation with estradiol and progesterone hormones. Copper and zinc have a significant correlation with reproductive hormones mainly progesterone and estradiol (Prasad *et al*., 1989), which corroborated with the present study. Significant correlation was observed between serum copper and estradiol levels in goats of Kashmir valley, India (Yatoo *et al*., 2013) and positive correlation was observed between serum progesterone level and copper-zinc in cow and heifer throughout the oestrus cycle (Yildiz and Akar, 2001) and concurred with the present study. Deficiencies of copper have also been associated with retained placenta, embryonic death and decreased conception rates and anoestrus (Mudgal *et al*., 2014). The correlation among the minerals and hormones can be inferred by different ways. Calcium is involved in the synthesis of steroid hormones in ovaries and adrenal gland and the release of luteinizing hormone from the pituitary gland (Hurley and Doane, 1989).

Based on Logit analysis, it could be inferred that if estradiol and progesterone levels increased by one unit, the probability of the bovines to be healthy increases by 5 and 75 times, respectively. This indicated the importance of these hormones in alleviating the reproductive problems in animals. As zinc level increases by one unit, the probability of bovines to be devoid of reproductive problems by many folds (536 times) indicates the importance of zinc, especially in maintaining optimum level of hormone. Further, zinc plays a critical role in the reproductive hormones and immune status of animals (Capuco *et al*., 1990). Zinc is also an essential component of various enzyme systems involved in metabolism of carbohydrate, protein and nucleic acid metabolism, epithelial tissue integrity, cell repair and division, vitamin A and E transport and their utilization. A good zinc status also improved fertility by reducing lameness; cows show sign of oestrus and improved performance of bulls. Zinc has also been shown to increase plasma β-carotene level which is correlated to improvement in conception rates and embryonic development (Short and Adams, 1988).

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Conclusions

Mineral deficiency affects the hormone production and impairs the production potential of dairy animals resulting in reproductive disorders was evident in this study. The minerals that affect reproduction in bovines are mainly trace minerals and required in small amounts like copper and zinc determined from this study, although deficiencies of calcium and phosphorus can also affect the fertility in bovines. In overall, there is an inter-relationship between the hormones and minerals of dairy animals which determines the health status and reproductive performance of the dairy animals. Hence, supplementations of these microminerals to the dairy animals in these states are imperative to have better productivity of dairy animals.

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