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Effect of mineral and vitamins supplementation on hemato-biochemical parameters and reproductive performances of Gir and Jersey cross-bred cattle

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Abstract

The present study was conducted to assess the hematological, and biochemical parameters and their effects on reproductive performances during anoestrus conditions of Gir, and Jersey cross-bred heifer and cows on supplementation of minerals and vitamins. A total of 40 anoestrous cattle of 10 each under different categories such as Gir heifers, Gir cows, Gir X Jersey cross-bred heifers, and Gir X Jersey cross-bred cows with no corpus luteum in the ovaries and inactive ovaries, and the control and experimental group were confirmed as anoestrus. total erythrocyte count, hemoglobin concentration, packed cell volume, total leukocyte count, differential leukocyte count and glucose, total protein, cholesterol, calcium, magnesium, inorganic phosphorus, copper zinc, manganese, and iron were analyzed as hematological and serum biochemical parameters respectively. Logistic Regression (LR) was used to analyze the effect of vitamin and mineral supplements on the onset of estrus in cows using the SAS-9.2 software package. The levels of hemoglobin, PCV, TEC, TLC, neutrophil, eosinophil, lymphocyte, and monocyte was found to be statistically non-significant in the pre and post-medicated anoestrus animals. The level of serum glucose and zinc did not show any significant variation but the total serum protein, cholesterol, calcium, magnesium, phosphorus, copper, iron, and manganese were found at significantly higher levels ($p < 0.05$) in post-medicated anoestrus Gir heifers, Gir cows and Gir X Jersey cross-bred heifers. After supplementation of vitamins and minerals, the experimental anoestrus animals displayed the oestrus signs in different intervals. Out of a total of 24 animals, 14 numbers of cattle exhibited estrus within 7-14 days, and the rest of animals showed the signs of oestrus within 14 to 28 days. This study showed that 60% of heifers and cows were found to respond positively to the supplementation of vitamins and minerals.

Keywords: Cattle, cross-bred, Gir, Jersey, mineral, reproductive performances, serum biochemical parameters, and vitamins

Introduction

Animal husbandry and livestock are highly potential sectors of Indian Agriculture or the backbone of the Indian economy, especially of rural economy. It provides a livelihood for millions of families in the country. Worldwide dairy herd fertility has been stated to have declined drastically during the last decades leading to significantly increased calving intervals. The principal component of an increased calving interval is the prolongation of the interval between parturition and insemination. Anoestrus denotes a state of complete sexual inactivity with no manifestation of estrus and is observed during certain physiologic conditions, such as before puberty, during pregnancy, and lactation, and in seasonal breeders. It is most often a sign of permanent or temporary depression of ovarian activity, caused by seasonal changes in physical environment, nutritional deficiency, lactation stress, and aging. Certain pathological conditions of the ovaries or the uterus also suppress estrus manifestation. Several deficiency diseases and metabolic disorders result in delayed maturity and subsequent infertility in dairy cattle. To maintain high milk output, cows should give birth at the shortest possible intervals, by taking account of post-partum uterine involution, anoestrous, and the duration of pregnancy. These nutritional stresses can affect the reproductive system and may be manifested by delayed return to cyclicity and failure of the

animal to become pregnant. Energy, protein, vitamins, and minerals play a significant role in the establishment of fertility and among vitamins A, D, and E, and like micro minerals such as copper (Cu), manganese (Mn), iron (Fe), and zinc (Zn) are being recognized as a significant contributor for reproduction in dairy animals. Normal levels of biochemical constituents and mineral levels are of utmost importance for maintaining the functional integrity of the reproductive system^[1]. Changes in biochemical constituents and mineral levels are important indicators of physiological state^[2] as well as the reproductive performances of the animals^[3, 4]. Blood has crucial importance for the maintenance of physiological equilibrium in the body⁵. The knowledge of hematological values is useful in diagnosing various pathological and metabolic disorders, which can adversely affect the productive and reproductive performances of cows^[6]. Important reproductive disorders like anestrus and repeat breeding have adversely affected dairy industries⁷. Unlike other nutrients, living organisms cannot synthesize mineral elements and the availability of minerals also decreases with the maturity of fodder^[8] therefore, judicious mineral supplementation is essential to the livestock for better production and reproduction. Livestock production is often badly influenced by mineral deficiencies and imbalances of micro-nutrients. Trace elements including Cu, Fe, Zn, Mn, and certain macro-elements like Ca, Mg, and P are very essential for normal livestock growth. Trace elements may function as co-factors, as activators of enzymes, or as stabilizers of secondary molecular structure^[9]. Deficiency or excess of minerals like P, Cu, and Zn have been associated with subnormal fertility and anestrus conditions^[10]. A high incidence of anestrus is associated with deficiencies in glucose, protein, albumin, and globulin^[11]. The hydrolysis of cholesterol ester is essential for the synthesis of steroid-based sex hormones and its estimation may provide useful information on the functional status of the ovario-hypophyseal axis^[12]. Taking into consideration of above facts, the present study was undertaken to evaluate the reproductive performances and also to elucidate certain hematological parameters and biochemical profiles before and after supplementation of vitamins and minerals during anestrus conditions of Gir heifers, Gir X Jersey cross-bred heifers, Gir cows, and Gir X Jersey cross-bred cows in order

Materials and Methods

The present study was conducted at Haringhata Farm Complex, Mohanpur, Nadia West Bengal. A total of 40 anestrus cattle of 10 each under different categories such as Gir heifers, Gir cows, Gir X Jersey cross-bred heifers, and Gir X Jersey cross-bred cows were used. The animals with no corpus luteum in the ovaries and inactive ovaries were included in the anoestrus group. All the studied animals were 3 years and above of age, medium in size having 175 to 250 kg body weight. The animals were kept under standard farm management practices. Additionally, they were fed daily with 3-4 kg of dry roughage and 20-25 kg of green roughage with clean water. The selected cattle were divided into two major groups as experimental as group I and control as group II. The experimental group comprised twenty-four (24) animals and was subdivided into four groups of 6 in each group. The control group was comprised of sixteen (16) animals with a sub-group of 4 no animals from each category. All the animals in the control and experimental group were confirmed as anoestrus following 3 successive gynecological examinations at an interval of 7 days revealing the presence of poorly-developed genitalia

including small, soft, hard, smooth, and plane inactive ovaries with no corpus luteum.

Hematological analysis: The blood samples (20 ml) were collected from all animals aseptically and 5 ml of blood was transferred in each sterilized vial containing 10 mg of EDTA per ml as an anticoagulant for estimation of hematological profiles. The remaining blood (15 ml) was taken for separation of serum. The animals of the experimental group were treated with 5 ml of Vitamin A-D-E intramuscularly at weekly intervals for a month and 10 ml of 20% Sodium Salt of 4-Dimethylamino-2-Methyl Phenyl Phosphinic Acid intramuscularly at every alternate day for the same period. Additionally, 30 gm of mineral mixture was given orally to each experimental animal for one month. At the end of the supplementation period, the blood samples were collected for haemato-biochemical studies. The hematological parameters like total erythrocyte count (TEC), hemoglobin concentration (Hb), packed cell volume (PCV), total leukocyte count (TLC), and differential leukocyte count (DLC) were studied by standard methods^[13]. The contents of the above tube were mixed well and allowed to stand at room temperature for 5 minutes. The Optical Density (OD) of the test was measured and the Drabkin's solution (blank) at 540 nm was standardized on a digital colorimeter for analysis of hematological parameters. The packed cell volume, RBC, Hb%, and TLC were estimated by following standard procedure^[14]. Blood smears were made on clean and grease-free glass slides at an angle of 45°. The blood smears were stained using Leishman's stain. The slides were kept in the microscope and examined first at low power and then under oil emersion for counting of neutrophils, eosinophils, lymphocytes, and monocytes by tally marking.

Biochemical analysis: The serum biochemical parameters studied were glucose, total protein, cholesterol, calcium (Ca), magnesium (Mg), inorganic phosphorus (P), copper (Cu), zinc (Zn), manganese (Mn), and iron (Fe). Analysis of blood samples was carried out with reagents and procedures supplied along with the kits (Trans Asia Bio-Medicals Ltd., Solan, HP, India). Blood serum total protein was analyzed as per the standard protocols - Biuret Method, endpoint. Blood serum total protein was calculated as total protein (gm%) = Absorbance of Test / Absorbance of standard X concentration of std. (gm%). The concentration of blood serum cholesterol was estimated with reagents and procedures supplied along with the kits and analyzed as per the standard prescribed methods such as Dynamic Extended Stability, CHOD – PAP Method (With LCF), and End Point. The concentration of blood serum glucose was estimated with reagents and procedures supplied along with the kits and analyzed as per the standard prescribed methods – GOD – POD Method, End Point. Serum calcium was estimated by Atomic Absorption Spectrophotometer (Perkin Elmer A Analyst 100). The serum sample was diluted to 1:40 with a 0.1% (W/V) lanthanum chloride solution adjusted to ensure that concentrations fell within the suitable absorbance range and were expressed as mg/dl or mg%. Serum phosphorus was estimated by diluting at the rate of 1:6 with de-ionized water by an auto-analyzer with the help of a phosphorus Arsenazo kit (Diasis Diagnostic Sistemler, Turkey). Serum magnesium was estimated by Atomic Absorption

Spectrophotometer. The serum sample was diluted to 1:40 with a 0.1% (W/V) lanthanum chloride solution adjusted to ensure that concentrations fall within the suitable absorbance range. Serum samples were diluted to 1:6 with de-ionized water by using AAS (Perkin Elmer A Analyst 100).

Descriptive statistics was used to estimate the blood parameters of dairy cows and Logistic Regression (LR) was used to analyze the effect of vitamin and mineral supplements on the onset of estrus in dairy cows using the SAS-9.2 software package.

Results and Discussion

Hematological analysis: The mean hemoglobin level in the control and experimental group of pre-medicated and post-medicated anoestrus Gir and Gir X Jersey cross-bred heifers and cows showed no significant variation and the level of Hb% in anoestrus Kankrej cows were reported in the earlier studies¹⁵ and similar findings were also opined by other workers¹⁶ and reported that the hemoglobin concentration was within the normal range and it was non-significant after mineral supplementation (Table-1). The mean concentration of PCV (%) in the control group and experimental group of pre-medicated anoestrus Gir and Gir X Jersey cross-bred heifers and cows did not vary significantly. The level of PCV (%) was estimated at 38.03 ± 0.44 and 39.25 ± 0.30 in the experimental group of pre-medicated and post-medicated Gir heifers respectively. Further studies revealed levels of PCV (%) were 30.61 ± 0.70 and 32.8 ± 0.90 in the experimental group of pre-medicated and post-medicated Gir X Jersey cross-bred heifers respectively and the statistical analysis showed no significant variation between the experimental group of pre and post-medicated Gir X Jersey cross-bred heifers (Table-1) and Similar findings were reported by the earlier workers in anoestrus Kankrej heifers¹⁷.

The mean value of TEC ($10^6/\mu\text{l}$) in the control and experimental group of pre-medicated anoestrus Gir and Gir X Jersey cross-bred heifers and cows were almost similar and showed no significant variation between them. The mean value of TEC ($10^6/\mu\text{l}$) in the experimental group of pre-medicated and post-medicated Gir heifers was 4.81 ± 0.33 and 4.78 ± 0.09 respectively (Table 1). The statistical analysis showed no significant variation between the experimental group of pre and post-medicated Gir heifers. The insignificant variation in cows and buffaloes was also reported by other researchers in similar studies^{18, 19} and besides that RBC concentration was also documented as non-significant in a similar study¹⁶.

In the present study, the level of total leukocyte count showed that there was no significant variation in the level of TLC (cumm) between the control and experimental groups of pre-medicated anoestrus Gir and Gir X Jersey cross-bred heifers and cows. The mean value of TLC (cumm) in the experimental group of pre and post-medicated Gir heifers were 7000 ± 98.88 and 6895.83 ± 105.92 respectively (Table 1). The statistical analysis showed no significant variation between the experimental group of pre and post-medicated anoestrus Gir heifers and the present findings were supported by the findings of earlier research workers^{18, 20} in anoestrus Jersey cross-bred cows.

The mean value of neutrophil (%) in the control and experimental group of pre-medicated anoestrus Gir and Gir X Jersey cross-bred heifers and cows were almost similar and

showed no significant variation among them. The level of neutrophil (%) in the experimental group of pre-medicated and post-medicated Gir heifers was 31.66 ± 0.80 and 31.87 ± 0.60 . The statistical analysis showed no significant variation among the experimental group of pre and post-medicated Gir heifers and the parallel observations in anoestrus buffaloes were also documented¹⁹. The mean concentration of eosinophil (%) in the control group and experimental group of pre-medicated anoestrus Gir and Gir X Jersey cross-bred heifers and cows did not vary significantly. The levels of eosinophil (%) were estimated as $5 \pm 0.36\%$ and $5.29 \pm 0.23\%$ in the experimental group of pre-medicated and post-medicated Gir heifers, respectively and these findings showed no significant variation among the pre and post-medicated Gir heifers and the similar results were documented in anoestrus HF and Jersey cows²¹. The level of lymphocyte (%) among the control and experimental group of pre-medicated anoestrus Gir and Gir X Jersey cross-bred heifers and cows showed no significant variations but in the experimental group of pre-medicated and post-medicated Gir heifers were found to be 58.0 ± 0.68 and $58.25 \pm 0.50\%$ respectively. The experimental group of pre-medicated Gir X Jersey cross-bred anoestrus heifers and post-medicated Gir X Jersey cross-bred anoestrus heifers did not vary significantly and these observations in buffaloes were also reported earlier¹⁹. The level of monocyte with mean value (%) in the control and experimental groups of pre-medicated anoestrus Gir and Gir X Jersey cross-bred heifers and cows were almost similar and showed no significant variation (Table 1). The differences in monocytes in the experimental group of pre and post-medicated anoestrus Gir heifers were statistically non-significant and were found to be similar to those recorded in HF and Jersey cows^{20, 21}.

Serum biochemical analysis: The level of serum glucose (mg%) in the control and experimental group of pre-medicated anoestrus Gir and Gir X Jersey cross-bred heifers and cows were almost similar and showed no significant variation between them (Table-2) and nonsignificant variation among the pre and post medicated Gir heifers was also supported by the previous studies^{22, 23} in non-descript cows and buffaloes. The total serum protein (gm%) in the control group and experimental group of pre-medicated anoestrus Gir and Gir X Jersey cross-bred heifers and cows did not vary significantly. The concentration of serum total protein (gm%) in the experimental group of pre and post-medicated Gir heifers was 6.61 ± 0.17 and 6.90 ± 0.15 , respectively and it showed significant variation ($p < 0.05$) in the experimental group of anoestrus Gir heifers. The significantly higher level of serum total protein as observed in the experimental group might be due to medication of vitamins and minerals and therefore it showed significant variation ($p < 0.05$) between the pre and post-medicated Gir cows (Table-2) and the findings were parallel with the results of earlier studies²⁴. Further, the level of serum cholesterol (mg%) in the control and experimental group of pre-medicated anoestrus Gir and Gir X Jersey cross-bred heifers and cows were almost similar, and no significant variation was found among them. The concentration of serum cholesterol level slightly increased in experimental groups of post-medicated anoestrus Gir heifers and hence no variance was observed in pre and post-medicated anoestrus Gir heifers (Table-2) and earlier researchers have also

documented the alike findings in Jersey cross-bred cows [25]. [26]. The mean value of serum calcium (mg%) in the experimental group of pre and post-medicated anoestrus Gir heifers was 8.16 ± 0.243 and 8.82 ± 0.11 , respectively but the study showed a significantly higher concentration of calcium ($p < 0.01$) in post-medicated Gir heifers (Table-2) and therefore, minerals and vitamin-D supplementation played an important role in enhancing the serum Ca concentration in anoestrus animals [27, 28]. Besides this, the present study showed a significantly higher level of serum phosphorus ($p < 0.01$) in the experimental group of post-medicated anoestrus Gir heifers (Table-2) and this finding is parallel with the findings in the case of HF X Ongole Jersey cross-bred cows [29] and in Murrah buffaloes [30]. Further, the experimental group of pre-medicated and post-medicated Gir X Jersey cross-bred did not vary significantly [31]. The level of serum magnesium was 2.74 ± 0.10 and 2.86 ± 0.07 mg% in pre-medicated and post-medicated Gir cows, respectively and it showed a significantly higher level ($P < 0.01$) of serum magnesium (Table-2). And was no significant variation among the pre-medicated and post-medicated Gir X Jersey cross-bred cows [32]. The present study showed that there was no significant variation in the level of serum copper (ppm) among the control and experimental groups of pre-medicated anoestrus Gir and Gir X Jersey cross-bred heifers and cows. However, the level of copper (ppm) was significantly higher ($p < 0.05$) in post-medicated Gir heifers (Table-2), and highly significant serum copper in post-partum treated Jersey cross-bred cows was also observed [33]. The lower level of copper in the anoestrus group in the present study might be due to lower

estrogen levels in anoestrus animals but this study also showed no significant variation in terms of copper availability between the pre-medicated and post-medicated Gir X Jersey cross-bred heifers and similar observation in anoestrus Kankrej heifers was also reported by earlier workers [25]. The mean value of serum zinc (ppm) in the control and experimental group of pre-medicated Gir and Gir X Jersey cross-bred heifers and cows was almost similar and showed no significant variation among them. Further, the level of zinc (ppm) did not vary significantly among the pre-medicated and post-medicated Gir heifers. The mean concentration of serum zinc (ppm) showed no significant variation between the pre-medicated and post-medicated Gir X Jersey cross-bred heifers (Table 2). The present findings are parallel to the findings of earlier studies in Jersey cross-bred cows and buffaloes [34]. Significantly higher levels of serum zinc observed in post-medicated Gir cows might be due to the combined effect of vitamin and mineral supplementation on the fertility parameters and blood biochemical profiles.

Statistical analysis showed that the mean of serum iron was significantly higher ($p < 0.05$) in post-medicated Gir heifers. Further study revealed that the mean serum iron was significantly higher ($p < 0.05$) in post-medicated Gir X Jersey cross-bred heifers (Table 2). Similar findings were reported in post-partum treated Jersey cross-bred cows [33]. This study showed serum manganese significantly higher ($p < 0.05$) in post-medicated Gir heifers [35]. The mean value of serum manganese (ppm) showed no significant variation among the pre-medicated and post-medicated Gir X Jersey cross-bred heifers [34].

Table 1: Values represent mean $\pm$ SD of Hemoglobin, Packed cell volume, TEC, and TL

Parameters	Type of Animal	Control (n=4)	Experimental (n=6)	
			Pre-medication	Post-medication
Mean ($\pm$ SE) Hemoglobin (gm%)	Gir heifer	12.7 ± 0.12	12.68 ± 0.13	13.25 ± 0.11
	Gir x Jersey heifer	10.3 ± 0.17	10.45 ± 0.29	11.25 ± 0.26
	Gir cow	13.15 ± 0.25	13.21 ± 0.21	13.43 ± 0.16
	Gir x Jersey cow	10.75 ± 0.08	10.09 ± 0.36	11.56 ± 0.24
Mean ($\pm$ SE) Packed cell volume (%)	Gir heifer	38.15 ± 0.51	38.03 ± 0.44	39.25 ± 0.30
	Gir x Jersey heifer	29.7 ± 0.59	30.61 ± 0.70	32.8 ± 0.90
	Gir cow	37.5 ± 0.38	37.41 ± 0.75	39.36 ± 0.46
	Gir x Jersey cow	31.95 ± 0.69	32.13 ± 0.56	34.02 ± 0.59
Mean ($\pm$ SE) TEC ($10^6/\mu$ l)	Gir heifer	4.52 ± 0.12	4.81 ± 0.33	4.78 ± 0.09
	Gir x Jersey heifer	4.1 ± 0.05	4.45 ± 0.36	4.48 ± 0.10
	Gir cow	4.92 ± 0.11	4.78 ± 0.24	4.91 ± 0.07
	Gir x Jersey cow	4.32 ± 0.13	4.46 ± 0.51	4.77 ± 0.14
Mean ($\pm$ SE) TLC (cumm)	Gir heifer	7175 ± 165	7000 ± 98.88	6895.83 ± 105.92
	Gir x Jersey heifer	6625 ± 131	6600 ± 63.24	6420.2 ± 55.2
	Gir cow	7175 ± 118	7116 ± 263.83	7012.5 ± 170.8
	Gir x Jersey cow	6900 ± 254	6800 ± 82.45	6670.8 ± 115.3
Mean ($\pm$ SE) Neutrophil (%)	Gir heifer	31.75 ± 1.03	31.66 ± 0.80	31.87 ± 0.60
	Gir x Jersey heifer	33.0 ± 1.08	32.83 ± 0.70	32.95 ± 0.50
	Gir cow	33.5 ± 1.32	33.66 ± 0.80	33.5 ± 0.59
	Gir x Jersey cow	33.25 ± 0.85	32.66 ± 0.49	33.33 ± 0.39
Mean ($\pm$ SE) Eosinophil (%)	Gir heifer	5.50 ± 0.28	5.0 ± 0.36	5.29 ± 0.23
	Gir x Jersey heifer	4.25 ± 0.25	4.33 ± 0.21	4.37 ± 0.17
	Gir cow	5.25 ± 0.47	5.0 ± 0.25	4.95 ± 0.25
	Gir x Jersey cow	5.25 ± 0.25	5.0 ± 0.25	4.97 ± 0.19
Mean ($\pm$ SE) Lymphocyte (%)	Gir heifer	57.5 ± 1.44	58.0 ± 0.68	58.25 ± 0.50
	Gir x Jersey heifer	57.0 ± 1.22	57.33 ± 0.88	58.79 ± 0.61
	Gir cow	56.25 ± 1.03	56.66 ± 0.33	58.79 ± 0.42
	Gir x Jersey cow	56.0 ± 1.47	56.33 ± 0.95	58.20 ± 0.50
Mean ($\pm$ SE) Monocyte (%)	Gir heifer	4.75 ± 0.47	4.83 ± 0.40	5.05 ± 0.21
	Gir x Jersey heifer	5.0 ± 0.40	5.16 ± 0.40	5.0 ± 0.21
	Gir cow	4.75 ± 0.47	4.83 ± 0.75	4.72 ± 0.24

	Gir x Jersey cow	4.75±0.47	5.0±0.89	4.75±0.27
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Table 2: Mean (±SE) values of certain Biochemical Parameters of control and experimental groups

Parameters	Type of Animal	Control (n=4)	Experimental (n=6)	
			Pre-medication	Post-medication
Mean (±SE) values of serum glucose (mg%)	Gir heifer	36.98±2.17	37.18±1.06	38.13±1.33
	Gir x Jersey heifer	36.93±2.21	37.10±1.33	37.10±1.33
	Gir cow	36.75±2.20	36.68±1.81	37.73±1.23
	Gir x Jersey cow	36.92±2.21	36.96±0.40	38.15±1.16
Mean (±SE) total serum protein (gm%)	Gir heifer	6.55±0.23	6.61±0.17	6.90±0.15*
	Gir x Jersey heifer	6.0±0.29	6.06±0.32	6.06±0.27
	Gir cow	6.55±0.23	6.53±0.20	6.71±0.16*
	Gir x Jersey cow	6.41±0.23	6.78±0.16	6.92±0.16*
Mean (±SE) serum cholesterol (mg%)	Gir heifer	159.12±7.16	159.97±3.01	165.40±4.46
	Gir x Jersey heifer	131.30±6.27	129.7±4.84	134.3±4.42
	Gir cow	157.5±5.5	156.33±5.55	163.07±3.64
	Gir x Jersey cow	149.6±4.1	149.0±7.74	146.39±4.92
Mean (±SE) values of serum calcium (mg%)	Gir heifer	7.92±0.09	8.16±0.243	8.82±0.11**
	Gir x Jersey heifer	7.98±0.29	7.84±0.246	8.60±0.16**
	Gir cow	8.27±0.31	8.57±0.135	8.95±0.15*
	Gir x Jersey cow	7.87±0.16	7.99±0.193	8.75±0.19*
Mean (±SE) serum phosphorus (mg%)	Gir heifer	3.32±0.15	3.44±0.16	4.16±0.09**
	Gir x Jersey heifer	3.63±0.19	3.72±0.22	3.94±0.19
	Gir cow	3.52±0.22	3.62±0.14	4.02±0.12
	Gir x Jersey cow	3.85±0.43	3.58±0.24	4.02±0.06**
Mean (±SE) serum magnesium (mg%)	Gir heifer	2.54±0.08	2.58±0.08	2.80±0.06*
	Gir x Jersey heifer	2.57±0.06	2.41±0.07	2.65±0.05*
	Gir cow	2.54±0.08	2.74±0.10	2.86±0.07*
	Gir x Jersey cow	2.58±0.03	2.63±0.13	2.77±0.08
Mean (±SE) values of serum copper (ppm)	Gir heifer	0.43±0.03	0.46±0.02	0.56±0.02*
	Gir x Jersey heifer	0.48±0.06	0.49±0.04	0.53±0.03
	Gir cow	0.46±0.05	0.47±0.01	0.60±0.02**
	Gir x Jersey cow	0.40±0.04	0.43±0.01	0.56±0.01*
Mean (±SE) values of serum zinc (ppm)	Gir heifer	1.0±0.07	1.0±0.05	1.03±0.04
	Gir x Jersey heifer	1.03±0.04	0.82±0.04	0.98±0.04
	Gir cow	0.90±0.02	0.91±0.02	1.01±0.02**
	Gir x Jersey cow	0.81±0.01	0.80±0.03	0.84±0.12
Mean (±SE) values of serum iron (ppm)	Gir heifer	1.93±0.11	1.9±0.12	2.53±0.13*
	Gir x Jersey heifer	2.30±0.16	2.16±0.05	2.49±0.07*
	Gir cow	1.99±0.11	2.0±0.12	2.4±0.06*
	Gir x Jersey cow	2.29±0.10	2.3±0.07	2.69±0.09*
Mean (±SE) values of serum manganese (ppm)	Gir heifer	0.10±0.01	0.10±0.006	0.16±0.01**
	Gir x Jersey heifer	0.09±0.01	0.09±0.005	0.14±0.01
	Gir cow	0.11±0.006	0.11±0.005	0.16±0.01
	Gir x Jersey cow	0.09±0.002	0.08±0.005	0.14±0.007**

Mean (±SE) values of Biochemical Parameters such as glucose, TP, Cholesterol, Ca, P, Mg, Cu, Zn, Iron and Mn of control and experimental groups (pre-medication and post-medication) group of anestrous Gir and Gir X Jersey cross-bred heifers and cows. ** indicates significant at 1% level, * indicates significant at 5% level.

Effect of mineral and vitamin supplementation on reproductive performances: After supplementation of vitamins and minerals for 30 days to 24 experimental animals (Gir and Gir X Jersey cross-bred animals), 14 showed normal estrus symptoms. Four cattle (2 Gir heifers, 1 Gir X Jersey cross-bred heifer, and 1 Gir X Jersey cross-bred cow) exhibited estrus within 7-14 days after medication. However, 8 animals (2 Gir heifers, 3 Gir cows, 1 Gir X Jersey cross-bred heifer, and 2 Gir X Jersey cross-bred cows) exhibited estrus within 14 to 21 days and one Gir cow, one Gir X Jersey cross-bred cow also displayed the estrus within 21 to 28 days. The present study revealed that 60% of the heifers and cows were found to respond positively to the supplementation of vitamins and minerals, whereas 13% of the control groups of heifers and cows also showed estrus signs, and similar findings were also documented in the earlier studies [36, 37]. Earlier studies showed that Copper (Cu), Manganese (Mn), and Zinc (Zn)

through their superoxide dismutase enzyme activity play a significant role in ovarian steroidogenesis and luteal function. Phosphorous is found to be part of ATP and is synthesized from ADP and Pi. It is being utilized as a driving force for maintaining normal reproductive physiology [38] and previous studies also reported that Phosphorus (P) is associated with reproductive abnormalities in cattle and its deficiency induces anoestrus [12].

Conclusion

This study was based upon certain hematological and biochemical parameters and also to elucidate the reproductive performances of anoestrus cattle breeds including Gir heifers, Gir X Jersey cross-bred heifers, and Gir X Jersey cross-bred cows on the administration of vitamins and minerals. The results in terms of hematologic and biochemical analysis showed relatively similar findings

as suggested by the earlier workers. However, most of the hematological parameters revealed no significant level among experimental cattle groups. However biochemical analysis such as the total serum protein, cholesterol, calcium, magnesium, phosphorus, copper, iron, and manganese were found at significantly higher levels in post-medicated anoestrus cattle. This study established the variations in hematological and biochemical values manipulate reproductive physiology and hence, help diagnose the anoestrus conditions. Therefore, it is recommended that precise research should be conducted on detailed hematological and biochemical parameters to evaluate the reproductive performances of dairy cattle.

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