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Fertility Augmentation of Non-Descript Delayed Pubertal Cattle Heifers through Mineral Mixture Supplementation and Hormonal Intervention

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ABSTRACT

Normal productive and reproductive behavior in domestic animal is closely associated with interaction between hormones and nutritional status of the animal system. A double PG protocol and dietary mineral mixture supplementation were used on 24 non-descript delayed pubertal heifers, aged between 3-5 years to study the effect of both on reproductive performance. Heifers were randomly allocated into four equal groups, comprising A (Control), B, C & D treatment groups (n=6 in each group). All the groups were fed iso-nitrogenous and iso-caloric ration according to the body weight. Group A (n=6) was fed a ration containing 0% mineral mixture feeding on a daily basis. Group B (n=6) received rations containing a mineral mixture at 1% of the total diet per day. Group C (n=6) was subjected for double PG protocol and GnRH. Group D (n=6) was fed a ration that included a mineral mixture @ 1 % of the ration, along with the double PG protocol and the GnRH. Compared to group C, group D has slightly higher progesterone levels due to more pregnant heifers. A significant increase in plasma progesterone concentration was observed in the B, C, and D groups that received mineral mixtures and hormone protocols. A similar progesterone level was found in group C and group A at 60 days. The estrogen value in group D was lower due to the higher number of non-cyclic heifers in comparison to the other groups. Additionally, mineral concentrations in the treatment groups were significantly higher than those in the control group. Group A, B, C and D were recorded 33.3, 50.0, 66.6, and 83.3% pregnancy rate respectively after artificial insemination, which indicating the effect of combining hormonal protocol with mineral mixture supplementation.

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Introduction

Total cattle population in India is 193.46 million (Livestock census, 2019). Although such impressive figures exist, the average amount of milk produced by a cow is 2.3 liters, which is lower than the amount produced in developed countries. One of the reproductive constraints for Indian dairy cattle is delayed puberty, which adversely affects the production of milk. Puberty is the age in postnatal life when the gonads produce gametes and sex hormones in sufficient quantities to enable an animal to reproduce. The hypothalamic release of pulsatile gonadotrophin releasing hormone (GnRH) is considered the trigger for mammalian puberty, as it initiates the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) that are required for gonadal activity and gametogenesis. According to Fortes et al. (2016), puberty onset is influenced by many factors, including age, species, genotype, body weight, growth rate, and energy status. In the tropical condition the age at puberty in *Bos indicus* range between 16 and 40 months (Dowell et al., 1976). A particular body weight has a role in attainment of puberty and a low body weight causes delay in onset of puberty (Maquivar et al., 2006).

Venugopal and RamamohanRao (1982) reported that the serum levels of calcium of cyclic cows to be higher while that of anestrus cows. Calcium may have a role in steroidogenesis by influencing delivery or utilization of cholesterol by mitochondria or by stimulating the conversion of pregnenolone to progesterone (Shemesh and Shore, 1994). Anestrus cows have significantly lower calcium levels than normal cyclic cows, according to Kalita et al. (1999). During transition period, supplementing diets with high energy, trace minerals (Co, Zn, Se), and vitamin E significantly increased the postpartum fertility of crossbred cows in terms of postpartum estrus occurrence, service period, pregnancy rate, and service per conception (Balamurugan et al., 2018). Copper and zinc are involved in regulating progesterone production by luteal cells via involvement of superoxide dismutase (Sugino et al., 1999). Copper complexes with GnRH and interact with GnRH receptors and modulate intracellular signaling in the gonadotropic cells of the anterior pituitary (Michaluk and Kochman, 2007). Patra et al. (2001) investigated the effects of (GnRH) on heifers with smooth and quiescent ovaries during delayed maturation and post-pubertal anestrus. Studies have increasingly explored the interactions between nutrition, hormones, and altered reproduction in dairy cattle (Chagas et al., 2007; Sartori et al., 2010). The purpose of this study was to determine the mineral and hormonal profile of delayed pubertal cattle, as well as the effect of mineral mixture supplementation and hormonal intervention on reproductive performance.

Materials and Methods

Location

Present research was carried out in the Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science & Animal Husbandry, Rewa and different villages in and around Rewa (Madhya Pradesh, India).

Animal selection and design

Twenty-four non-descript delayed pubertal heifers of Rewa district, aged between 3-5 years were selected to study the effect of dietary mineral mixture supplementation and double PG protocol on biochemical changes and reproductive performance (Table 1 and 2). Heifers were randomly allocated into four equal groups, comprising three treatment groups (n=6 in each) and control (n=6) group. The experimental animals were maintained under an intensive housing management system. Before the commencement of study, proper health care including vaccination & deworming were taken into consideration. Heifers in the treatment group were fed concentrate feed supplemented with a mineral mixture on the ration basis. Control group was fed ration containing same CP and energy level according to the body weight to make the diet isonitrogenous and isocaloric.

Table 1: Experimental design for mineral mixture supplementation and hormonal intervention to all four groups

Groups	Treatment Regimen			Artificial insemination	Pregnancy diagnosis
	z	1st inj Cloprostenol	2nd inj Cloprostenol		
A (Control, n=6)	-	-	-	Depend on estrus	60 days post AI
B (Treatment, n=6)	@ 50 gm/cow/ day for 60 days	-	-	Depend on estrus	60 days post AI
C (Treatment, n=6)	-	1st day 2ml I/M	11th day 2ml I/M	After 48hrs of 2nd inj Cloprostenol with Buserelin inj 2.5 ml. I/M	60 days post AI
D (Treatment, n=6)	@ 50 gm/cow/ day for 60 days	1st day 2ml I/M	11th day 2ml I/M	After 48hrs of 2nd Inj Cloprostenol with Buserelin inj 2.5 ml. I/M	60 days post AI

Table 2: Composition of mineral mixture supplement

Ingredient	Quantity	Ingredient	Quantity
Vitamin A	7,00,000 IU	Iodine	325 mg
Vitamin D	70,000 IU	Vitamin E	250 mg
Zinc	960 mg	DL-Methionine	1000 mg
Magnesium	600 g	Cobalt	150 mg
Manganese	1500 mg	Potassium	100 mg
Iron	1500 mg	Sodium	5.9 mg
Copper	1200 mg	Calcium	25.5 %
Nicotinamide	1000 mg	Phosphorus	12.75 %
Sulphur	0.72 %		

Blood sampling and hormone assay

Blood samples were collected by jugular venipuncture on day 0, 7, 14, 21, 42 and 60 during feeding trials. (Ethical approval letter no. 05/IAEC/Vety/Rewa/2019 dated 04/11/2019). Serum progesterone concentration (ng/ml) and estrogen (pg/ml) were estimated on the day of treatment 0, 7, 14, 21, 42 and 60 day after the start of treatment for confirming the result obtained by gyneco--clinical examination. An analysis of progesterone and estrogen concentrations was also conducted to determine how the treatment affected fertility. The quantitative determination of progesterone concentration in serum was performed by ELISA using kits supplied by Cayman chemicals, USA. Estrogen in serum samples was estimated by RIA technique using diagnostic I^{125} kits supplied by Immunotech, France and BARC, Mumbai.

Mineral profile

Using standardized kits, zinc, iron, copper, cobalt, and selenium were measured by Atomic Absorption Spectrophotometer, whereas calcium, phosphorus, and magnesium were measured by calorimetry. A mineral deficit/excess/imbalance was calculated by comparing the mineral availability of individual animals with the mineral requirements in feeding standards (Kearl, 1982; ICAR, 1998).

Reproductive performance

The onset of estrus in heifers was detected by cervical-vaginal discharge and per-rectal examination. Pregnancy diagnosis in cows of all the groups was performed on day 60 post insemination by rectal palpation. Reproductive outcome such as onset of estrus and pregnancy diagnosis were observed in all experimental cattle.

Statistical analysis

The data obtained were subjected to analysis of variance using IBM®SPSS software version 22 statistical package. Data from different experiments are presented as mean±SE. The pairwise comparison of means was carried out using Tukey's multiple comparison tests. The difference at $P \leq 0.05$ was statistically significant.

Results and Discussion

Serum progesterone

The mean concentrations of serum progesterone from day 0 to 60 in groups (A) and treatment group (B, C and D) have been presented in Table 3. The progesterone level significantly varies ($P \leq 0.05$) at 21 days and at 60 days. The progesterone level significantly varies ($P \leq 0.05$) at 21 days and at 60 days. The progesterone level significantly varies ($P \leq 0.05$) between 14 and 60 days. At day 14, 21 and has similar value but significantly different ($P \leq 0.05$) compared to day 0, 7, 42 and 60. The progesterone level significantly varies ($P \leq 0.05$) between 14 and 60 days. At day 14, 21 and has similar value but significantly different compared to day 0, 7, 42 and 60. Serum progesterone concentration of B, C and D Group, which supplemented with mineral mixture and hormonal protocol were significantly high ($P \leq 0.05$).

The progesterone concentration is responsible for stimulation of cyclic, follicular development and also for continuation of pregnancy. In normal cyclic animals, the serum progesterone level is expected to be high during diestrus stage and subsequently should reduce during estrus. Anestrus cases carry irregular levels. The findings of serum progesterone concentrations (ng/ml) are similar to the finding in dairy cow (Ayres et al., 2013). Khade et al. (2011) reported the mean plasma progesterone concentration 1.7±0.28 ng/ml in non-conceived cows. In the present work, progesterone values are in close agreement with Devasena et al. (2010). Compared to not supplementing, the profile of progesterone increased after supplementation because of the improvement in estrus and conception rate.

Table 3: Estimation of serum progesterone (ng/ml) at various days (mean±SEM) in all the experimental groups.

Groups	Concentration (ng/ml) of serum progesterone at various days (mean±SEM)					
	0	7	14	21	42	60
A	0.06±0.00	0.08±0.01	0.14±0.03	0.21±0.07	1.19±0.67	1.49±0.86
B	0.09±0.02	0.14±0.04	0.20±0.05	0.76±0.53	1.39±0.73	2.37±0.96
C	$0.13±0.04^{a}$	$0.23±0.07^{a}$	$1.24±0.66^{ab}$	$1.53±0.78^{ab}$	$2.81±0.85^{ab}$	$3.16±0.93^{b}$
D	$0.15±0.04^{a}$	$0.23±0.06^{a}$	$0.83±0.53^{ab}$	$1.51±0.71^{ab}$	$2.98±0.82^{bc}$	$4.33±0.21^{c}$

Different superscripts in small letter (a, b, c, d) in a row differ significantly (P ≤ 0.05).

Table 4: Estimation of serum estrogen (pg/ml) at various days (mean±SEM) in all the experimental groups.

Groups	Concentration (pg/ml) of serum estrogen at various days (mean±SEM)					
	0	7	14	21	42	60
A	4.27±0.42	4.70±0.43	5.33±0.88	7.91±3.14	7.73±2.50	5.16±0.35
B	4.85±0.31	5.59±0.32	8.65±2.63	5.25±0.43	7.54±2.69	8.55±3.15
C	$4.60±0.43^{a}$	$5.32±0.43^{a}$	5.43±0.26	$6.01±0.49^{ab}$	$5.05±0.23^{ab}$	$4.61±0.19^{b}$
D	$5.65±0.23^{a}$	$5.48±0.49^{a}$	5.89±0.24	$8.39±2.45^{ab}$	$5.28±0.38^{bc}$	$4.40±0.09^{c}$

Different superscripts in small letter (a, b, c, d) in a row differ significantly (P ≤ 0.05).

Serum Estrogen

The mean concentration of serum estrogen from day 0 to 60 in group A and treatment group (B, C and D) have been presented in Table 4.The estrogen level significantly varies (P ≤ 0.05) and observed higher at 21 days and low at 60 days. The estrogen level significantly varies (P ≤ 0.05) between 7 and 42 days. At 7-day group A and B and also group C and D has similar values but significantly different (P ≤ 0.05) from other groups. Estrogen is produced by the follicles, which located on the ovary, as the follicle grows; more estrogen is producing (Mondal et al., 2019). Estrogen act in positive feedback mechanism and responsible for LH surge and ovulation, it also affects the nervous system of cow causing restlessness, phonation mounting and most importantly, the willingness to be mounted by other animals (Jena et al., 2016). The present findings show similar results as in study of Bhowmik et al. (2014) and Michaluk and Kochman (2007).

Serum Calcium

The mean concentration of serum Calcium from day 0 to 60 in groups (A) and treatment group (B, C and D) has been presented in Table 5. The Calcium level significantly varies (P ≤ 0.05) between 0 and 60 days.

Sahoo et al. (2017) and Virmani et al. (2011) reported a range of calcium (mg/dl) in anestrous cattle 7. 94±0. 08 and 7. 50±1. 21, respectively in anestrous cattle. Several studies have supported the present study, such as Akhtar et al. (2009) and Tewari et al. (2013). The present study shows that the better result was obtained if animals are supplemented with a mineral mixture have better Ca value, then control Group.

Serum Magnesium

The mean concentrations of serum Magnesium from day 0 to 60 in all the groups have been presented in Table 6. The Magnesium level significantly varies (P ≤ 0.05) between 0 and 60 days. The present values are within the normal range and in close agreement with Das et al. (2002), Gowda et al. (2001) and Tiwary et al. (2007).

Serum Copper

The mean concentration of serum copper from day 0 to 60 in group (A) and treatment group (B, C and D) have been presented in Table 7. In the group C, copper level significantly varies (P ≤ 0.05) between 0 and 60 days. On day 0, 21, 42, 60, copper level significantly varies (P ≤ 0.05) among various groups. The findings of Kalita et al. (1999) and Dutta et al. (2001) are similar to these present findings in heifers.

Serum Zinc

The mean concentration of serum Zinc from day 0 to 60 in group (A) and treatment group (B, C and D) have been presented in Table 8. Group D has significantly high (P ≤ 0.05) Zinc levels in comparison to other groups. The present study is corroborated with the finding of Das et al. (2003). The findings of this study were in agreement with

Table 5: Estimation of serum Calcium (mEq/L) at various days (mean±SEM) in all the experimental groups.

Groups	**Concentration** (mEq/L) **of serum Calcium at various days (mean±SEM)**			
	0	**21**	**42**	**60**
A	3.87±0.22	3.85^{A}±0.17	3.84^{A}±.012	3.95^{A}±0.11
B	3.71±0.03^{a}	3.93^{aA}±0.06	4.22^{abAB}±0.04	4.43^{bB}±.048
C	4.11±0.21^{a}	4.20^{abAB}±0.19	4.41^{bcBC}±0.18	4.58^{bcBC}±0.17
D	4.31±0.26^{a}	4.50^{bB}±0.22	4.74^{cC}±0.20	4.99^{cC}±0.18

Different superscripts in small letter (a, b, c, d) in a row and capital letter (A, B, C, D) in a column differ significantly ($P \le 0.05$).

Table 6: Estimation of serum Magnesium (mEq/L) at various days (mean±SEM) in all the experimental groups.

Groups	**Concentration** (mEq/L) **of serum Magnesium at various days (mean±SEM)**			
	0	**21**	**42**	**60**
A	0.95^{a} ±0.11	1.36^{b}±0.09	1.62^{c}±0.06	1.88^{dB}±0.04
B	1.00^{a}±0.14	1.41^{b}±0.08	1.64^{b}±0.07	1.91^{cB}±0.03
C	1.11^{a}±0.18	1.29^{ab}±0.17	1.53^{ab}±0.11	1.68^{bA}±0.10
D	0.94^{a}±0.15	1.42^{b}±0.05	1.71^{c}±0.04	1.94^{cB}±0.01

Different superscripts in small letter (a, b, c, d) in a row and capital letter (A, B, C, D) in a column differ significantly ($P \le 0.05$).

Table 7: Estimation of serum Copper (μmol/L) at various days (mean±SEM) in all the experimental groups.

Groups	**Concentration** (μmol/L) **of serum Copper at various days (mean±SEM)**			
	0	**21**	**42**	**60**
A	5.96^{aA}±0.27	6.72$^{a A}$±0.45	7.22^{aA}±0.35	7.79^{aA}±0.11
B	6.28^{abAB}±0.147	8.27^{bB}±0.22	9.97^{bB}±0.26	12.22^{bB}±0.40
C	7.40^{cC}±0.38	9.21^{cBC}±0.58	10.86^{bcBC}±0.92	12.36^{bB}±1.25
D	7.01^{bcBC}±0.22	9.84^{cC}±0.25	11.94^{cC}±0.33	14.74^{cC}±0.26

Different superscripts in small letter (a, b, c, d) in a row and capital letter (A, B, C, D) in a column differ significantly ($P \le 0.05$).

Table 8: Estimation of serum Zinc (μmol/L) at various days (mean±SEM) in all the experimental groups.

Groups	**Concentration of** (μmol/L) **serum Zinc at various days (mean±SEM)**			
	0	**21**	**42**	**60**
A	5.91^{aA}±0.30	6.73^{aA}±0.35	7.27^{aA}±0.30	7.95^{aA}±0.31
B	6.45^{bcBC}±0.18	8.42^{bB}±0.19	10.20^{bB}±0.31	12.27^{bB}±0.28
C	7.37^{cC}±0.47	9.35^{bcBC}±0.58	10.87^{bcBC}±0.88	12.25^{bB}±1.22
D	7.13^{cC}±0.22	9.96^{cC}±0.32	11.95^{cC}±0.28	14.60^{cC}±0.26

Different superscripts in small letter (a, b, c, d) in a row and capital letter (A, B, C, D) in a column differ significantly ($P \le 0.05$).

Joy and Nair (1995) and Singh et al. (2005), as they also observed non- significant difference in between anestrus and cyclic cows.

Serum Phosphorus

The mean concentrations of serum phosphorus from day 0 to 60 in groups (A) and treatment group (B, C and D) have been presented in Table 9. Group B and D have significantly high ($P \le 0.05$) Phosphorus levels compared to other groups. The higher level of phosphorus than the present study in delayed puberty was observed by Shrivastava and Kadu (1995) and Singh et al. (2005).

Serum Cobalt

The mean concentrations of serum Cobalt from day 0 to 60 in groups (A) and treatment group (B, C and D) have been presented in Table 10. Prasad et al. (1989) determined serum levels of cobalt in prolonged post-partum anestrus cows as 1.05 ± 0.3 μg/ml and 0.5 ± 0.1 μg/ml in acyclic cows (smooth ovaries). These findings were found to be lower than the findings of the present study.

Table 9: Estimation of serum Phosphorus (mEq/L) at various days (mean±SEM) in all the experimental groups.

Groups	Concentration (mEq/L) of serum Phosphorus at various days (mean±SEM)			
	0	21	42	60
A	1.73±0.09	1.89^{aA}±0.16	2.09^{aA}±0.12	2.36^{aA}±0.07
B	2.01±0.16	2.49^{bB}±0.182	2.99^{bC}±0.16	3.36^{cB}±0.15
C	1.97±0.11	2.16^{abAB}±0.11	2.35^{abAB}±0.18	2.64^{aA}±0.15
D	1.83±0.07	2.35^{abAB}±0.15	2.74^{bcBC}±0.16	3.21^{cB}±0.19

Different superscripts in small letter (a, b, c, d) in a row and capital letter (A, B, C, D) in a column differ significantly (P ≤ 0.05).

Table 10: Estimation of serum Cobalt (ppm) at various days (mean±SEM) in all the experimental groups.

Groups	Concentration of serum Cobalt (ppm) at various days (mean±SEM)			
	0	21	42	60
A	0.051±0.003	0.06±0.003	0.08^{abAB}±0.003	0.10^{aA}±0.010
B	0.046±0.003	0.07±0.003	0.09^{bB}±0.012	0.13^{bB}±0.014
C	0.050±0.005	0.06±0.006	0.06^{aA}±0.004	0.07^{aA}±0.003
D	0.041±0.004	0.06±0.003	0.08^{abAB}±0.003	0.13^{bB}±0.010

Different superscripts in small letter (a, b, c, d) in a row and capital letter (A, B, C, D) in a column differ significantly (P ≤ 0.05).

Table 11: Estimation of serum Selenium (ppm) at various days (mean±SEM) in all the experimental groups.

Groups	Concentration of serum Selenium (ppm) at various days (mean±SEM)			
	0	21	42	60
A	0.041±0.003	0.055±0.005	0.066^{A}±0.006	0.080^{A}±0.04
B	0.045±0.004	0.066±0.003	0.083^{B}±0.003	0.120^{B}±0.09
C	0.043^{a}±0.003	0.060^{b}±0.005	0.066^{bA}±0.006	0.076^{Ba}±0.05
D	0.048^{a}±0.003	0.068^{a}±0.003	0.086^{bB}±0.002	0.138^{bB}±0.12

Different superscripts in small letter (a, b, c, d) in a row and capital letter (A, B, C, D) in a column differ significantly (P ≤ 0.05).

Table 12: Estimation of serum Iron (µmol/L) at various days (mean±SEM) in all the experimental groups.

Groups	Concentration of serum Iron (µmol/L) at various days (mean±SEM)			
	0	21	42	60
A	11.52^{aA}±0.49	14.44^{aA}±0.78	17.42^{bA}±1.31	20.23^{bA}±1.76
B	14.18^{aB}±0.81	19.17^{bB}±0.98	24.46^{cC}±1.18	29.14^{cC}±1.19
C	15.05^{aB}±0.67	18.06^{bB}±0.67	20.92^{bB}±0.64	24.35^{cB}±0.74
D	14.23^{aB}±0.85	20.05^{bB}±0.49	25.28^{cC}±0.68	30.09^{cC}±0.60

Different superscripts in small letter (a, b, c, d) in a row and capital letter (A, B, C, D) in a column differ significantly (P ≤ 0.05).

Serum Selenium

The mean concentrations of serum Selenium from day 0 to 60 in groups (A) and treatment group (B, C and D) have been presented in Table 11. The Selenium level significantly varies (P ≤ 0.05) between 0 and 60 days. At 60-day selenium level significantly varies (P ≤ 0.05) between groups. Selenium increases fertility in cattle by reducing the incidence of anestrus (Hidiroglou, 1979; Harrison et al., 1984).

Serum Iron

The mean concentrations of serum Iron (µmol/l) from day 0 to 60 in groups (A) and treatment group (B, C and D) have been presented in Table 12. On different days and between different groups, the iron level significantly varies (P ≤ 0.05) between 0 and 60 days. The higher plasma iron concentration in anestrus cows than the level observed in the present study was reported by Tambe et al. (1996), Kalita et al. (1999) and Dutta et al. (2001). However, lower level of plasma iron concentration in anestrus cows was observed by Vohra et al. (1995) and Ramakrishna (1997).

Conception Rate

According to the present study, conception rates in non-descript delayed pubertal heifers were 33.3% (n=6), 50.0% (n=6), 66.6% (n=6) and 83.3% (n=6) in groups A, B, C, and D respectively. Combining hormonal protocol with mineral mixture supplementation improved conception rate. Similarly, Yasothai (2014) and Devasena et al. (2015) reported similar results with mineral mixture supplementation. The hormonal protocol values obtained are consistent with those obtained by Kumar (2014) and Balamurugan et al. (2018).

Conclusion

Mineral mixture supplementation and hormonal intervention such as double PG and GnRH administration resulted in higher fertility in delayed pubertal non-descript heifers; group D showed a higher conception rate as a result of mineral supplementation as compared to group A, B, and C.

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