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Biochemical effect of Heatsynch protocol with mineral mixture supplement and bypass fat on postpartum anoestrous crossbred cows

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Abstract
The present investigation was conducted to study the biochemical effect of Heatsynch protocol with mineral mixture (nutritional supplementation) and bypass fat on postpartum anoestrous crossbred cows. Twenty postpartum anoestrous crossbred cows were selected based on the history and rectal palpation in Kamrup district of Assam, India. The animals were divided into two groups, Group A and Group B i.e. ten animals in each group. The animals in Group A received Heatsynch + mineral mixture + bypass fat and AI was done at induced oestrus. Group B animals were kept as control without any treatment. Blood samples were collected from cows on day 0, 7, 8 and 9 of Heatsynch protocol for the estimation of serum biochemical profiles. The use of mineral mixture + bypass fat + Heatsynch protocol resulted in 100.00 per cent oestrus induction with mean interval of 32.60 ± 1.54 hours. The conception rate at induced oestrus was found to be 60.00 per cent. The mean serum oestrogen, serum cortisol, total serum protein, serum calcium, serum phosphorus and serum zinc on day 0, 7, 8 and 9 revealed that the values on 9th day were significantly higher than the values obtained on day 0, 7 and 8. The mean serum progesterone and serum cholesterol concentration was maximum on day 7 and 0 respectively. Based on the investigation it was found that Heatsynch protocol with nutritional supplementation and bypass fat resulted in effective induction of oestrus with satisfactory conception rate in postpartum anoestrous crossbred cows.

Keywords: Biochemical, Bypass fat, Cow, Crossbred, Heatsynch, Mineral mixture & Postpartum anoestrous

Introduction
Anoestrus is one of the most common forms of infertility in cattle in Assam. Post partum anoestrus is one of the major obstacles in livestock production leading to prolonged inter calving period, delayed conception, reduction in milk yield and number of calves during life time which cause high economic loss to the farmers and proved to be challenging problem to the veterinarians as well. Prolonged postpartum anoestrus condition is characterized by the complete absence of ovarian activity which possibly may be due to the lack or deficiency in release of FSH, LH and other hormones. Insufficient LH secretion associated with energy intake has been reported to be one of the major causes of postpartum anoestrus in cattle. It is also clear that prolonged postpartum anoestrus is due to failure of dominant follicle to ovulate rather than delay in the development (Butler, 2000) [10]. Deficiency of minerals especially calcium, phosphorus and zinc upsets the proper functioning of reproductive organs due to poor follicular development and may led to postpartum anoestrus in cows. This condition is collectively imposed by poor nutrition along with lactation stress suppressing reproductive functions. During the last few years, several attempts have been made to treat the prolonged postpartum anoestrus in cows by using hormones for successful induction of oestrus in anoestrous cows. Postpartum anoestrus in cattle was reported to be corrected by strategic supplementation of mineral mixture (Jana *et al.*, 2015) [4]. So, keeping in view of these, the present study was done to investigate the biochemical effect of Heatsynch protocol with nutritional supplementation and bypass fat on postpartum anoestrous crossbred cows.

Materials and Methods
The present investigation was conducted to study the biochemical effect of Heatsynch protocol with mineral mixture and bypass fat on postpartum anoestrous crossbred cows.

Twenty (n=20) numbers of crossbred cows with an extended period of postpartum anoestrus (>90 days), free from reproductive abnormalities and systemic diseases were selected as experimental animals for the present study. The animals were divided into two groups, Group A and Group B i.e. ten animals in each group. The animals in Group A received supplemented with mineral mixture (VM ^{ALL}) @ 30 g/animal/day and bypass fat @ 100 g/animal/day for a period of 30 days and 20 days, respectively followed by Heatsynch protocol i.e. 5 ml GnRH (Receptal VET) injection on 0 day intravenously, 2 ml PGF2α (Estrumate) injection on 7th day intramuscularly, 1ml Estradiol Benzoate (Pregheat) injection on 8th day intramuscularly and 5 ml GnRH injection on 9th day intravenously. Group B animals were kept as control without any treatment. Blood samples were collected from cows on day 0, 7, 8 and 9 for the estimation of serum biochemical profiles. Collected blood samples were transferred to sterilized clot activator (eVAC Tube) vial and kept in slanting position for clotting. After removal of the clot, samples were subjected to centrifugation @ 1500 rpm for 10-15 minutes. Separated serum samples were transferred to micro centrifuge tubes and stored at -20 °C until analysis. Level of oestrogen, progesterone and cortisol in serum sample was estimated by solid phase competitive ELISA method. Serum cholesterol level and total serum protein concentration was estimated by using cholesterol Kit and Total Protein Kit respectively. The biochemical tests for Calcium, Phosphorus and Zinc were done by using respective Kits. Artificial insemination was performed in cows that came to oestrus after treatment. Cows were subjected to rectal palpation for diagnosis of pregnancy after three months of insemination. Lastly, statistical analysis (ANOVA and t Test) was carried out with the help of standard software (SPSS2O).

Results and Discussions

Response to Treatment by the Postpartum Anoestrous Crossbred Cows

From Table 1 it was found that, the use of mineral mixture + bypass fat + Heatsynch protocol in grouop Group A resulted in 100.00 per cent oestrus induction with mean interval of 32.60 ± 1.54 hours (26 to 45 hrs). The mean duration of oestrus was 22.80 ± 0.62 (19 to 25 hrs) and the conception rate at induced oestrus was found to be 60.00 per cent. None of the cows in group B exhibited oestrus during the study period. The higher oestrus in Group A might be due to mineral supplementation to the animals prior to hormonal treatment. Minerals have a beneficial role in endocrine system and play an important role in resumption of follicular growth. Bypass fat and mineral mixture might have showed a beneficial effect on postpartum ovarian activity by increasing the number of ovarian follicles and enhancing follicular growth (Colazo *et al.,* 2009) [9]. The oestradiol benzoate used in the in the present study might have influenced on the duration of oestrus, since there is a linear relationship between the duration of oestrus and level of estrogen. Minerals are known to enhance the reproductive efficiency greatly in postpartum anoestrous cows as they function as co-enzymes of various metabolic functions particularly in production of steroid hormones. Moreover, dietary fats may have considerable effect on reproduction because fatty acids and cholesterol are substrates for steroid hormone synthesis. So, feeding high fat diets to postpartum anoestrous cows increased follicular growth with more progesterone production and lengthened the lifespan of the corpus luteum. Further, nutritional supplement and hormonal treatment might have improved resumption of ovarian activity and subsequent conception rates as they maintained the energy balance and body reserve which helped in enhancing reproductive performance (Butler, 2000) [10].

Table 1: Response to Treatment by the Postpartum Anoestrous Crossbred Cows (Group A)

S. No	Parameters	Group A (N=10)	Group B (N=10)
1	Oestrus response (%)	100.00 (10/10)	-
2	Interval between treatment and onset of induced oestrus (hrs)	32.60a ± 1.54	-
3	Duration of oestrus (hrs)	22.80 ± 0.62	-
4	Conception rate (%)	60.00 (6/10)	-

Estimation of Blood Bio Chemicals

The value of mean serum oestrogen, progesterone, cortisol, cholesterol concentration, total serum protein, calcium, phosphorus and zinc of group A and group B are given in table 2.

Level of serum oestrogen

In the present study, it was observed that the serum oestrogen concentration was significantly higher on day 9 in Group A (18.33±0.30 pg/ml) as compared to the corresponding values recorded on day 0, 7 and 8. The level of oestrogen within the treatment Group A varied significantly on different days of treatment with an exception of control group where estrogen level did not vary significantly. Level of oestrogen before heatsynch protocol was low, which might be due to ovarian inactivity of the animals. First GnRH injection might have caused ovulation of dominant follicle if present and if not luteinized follicles with emmergance of new follicular waves 1-2 days later resulting in gradual increse in estrogen level. Highest level of oestrogen was recorded on day 9 which might be attributed to injection of oesteadiol benzoate on day 8. Similar finding were also reported by Jana *et al.* (2015) [4], Sahoo *et al.* (2016) [6] and Bhutani *et al.* (2009) [1].

Level of Serum progesterone

Table 2 showed that, in Group A, the serum progesterone concentrations was significantly higher on day 7 (2.69 aB±0.01ng/ml) and varied significantly within the group on different days of treatment. Variation in the serum progesterone levels in the present study might be due to the status of the animals. Low level of progesterone on day 0 of heatsynch protocol might be due to ovarian inactivity of the animals. Gradual increase in serum progesterone level upto day 7 might be due to CL formation or luteinisation of follicles present in the ovary following first GnRH inj. Significant reduction in serum progesterone levels on day 8 and 9 might be due parenteral administration of PGF2α, and oestradiol benzoate and caused physiological declination of progesterone towards the basal level for manifestation of oestrus. This finding was in conformity with that of Mohapatra *et al.* (2012) [5], Jana *et al.* (2015) [4] and Sahoo *et al.* (2016) [6].

Level of Serum cortisol

It was also observed that in Group A, the serum cortisol was significantly higher on day 9 (6.78aD ± 0.07 ng/ml) and varied significantly within the group on different days of treatment. Similar finding was also reported by Sikka *et al.* (1993) [7] and was in corroboration with the present findings. However, Singh *et al.* (2015) [8] reported higher cortisol level

(9.42±1.33 ng/ml) in fat supplemented anoestrus cows. Variation in the serum cortisol levels might be due to variation in the environment, stress factor and level of nutrition (Sikka *et al.*, 1993) [7].

Level of serum cholesterol

The serum cholesterol concentrations was found to be significantly higher on day 0 (119.96b ± 0.67 mg/dl) in Group A and on day 9 (112.85c ±1.05 mg/dl) in group B. There was a huge significant difference in the serum cholesterol level between the groups. However within both the groups, the level of cholesterol did not vary significantly. The higher level of serum cholesterol in group A might be attributed to the feeding of bypass fat in diet prior to heatsynch protocol. Cholesterol serves as the precursor of steroid hormone (progesterone) synthesis in ovarian luteal tissues. Increased concentration of cholesterol from fat supplementation might lead to an increase progesterone synthesis after ovulation or reduced rate of clearance from blood, thus helping in implantation of embryo and maintaining pregnancy (Bhutani *et al.*, 2009) [1].

Level of total serum protein

The mean level of total serum protein in postpartum anoestrous crossbred cows on different days of treatment in Group A was found to be highest on day 9 (8.00b ± 0.13 g/dl) and without treatment in Group B was on day 8 (6.92a ± 0.13 g/dl) with no significant variation. The higher level of total serum protein in group A might be due to supplementation of bypass fat prior to heatsynch protocol. Lower concentration of protein in blood was reported to be associated with anoestrus in cow, which could be due to reduced functioning of pituitary gland and reproductive organs (Bhutani *et al.*, 2009) [1]. Protein deficiency caused deficiency of certain amino acids required for synthesis of gonadotropins thus caused reproductive disturbances. Production of high quantity of milk that required higher level of protein might affect reproduction in postpartum cows (Buhecha, 2015) [3].

Level of serum calcium

The mean level of total serum calcium was found to be higher in Group A than Group B and was found to be maximum on day 9 (8.66a ±0.03 mg/dl). Variation of serum calcium level in postpartum anoestrous cows might be consequential to drainage of calcium during lactation, since calcium played a vital role in GnRH stimulation for the release of LH from pituitary cells, the concentration of which is required to rise for approaching oestrus. Release of LH was known to be prevented in absence of calcium or in the presence of calcium blocking agents. Low level of calcium was found to be associated with reduced steroidogenesis in the ovaries leading to the condition of anoestrus in cows (Bindari, 2013) [2].

Level of serum phosphorus

The mean level of serum phosphorus in postpartum anoestrous crossbred cows on different days of synchronization protocol was 4.16 ± 0.03, 4.19 ± 0.02, 4.21 ± 0.02 and 4.22 ± 0.03 (mg/dl) in group A and were higher than the Group B animals' mean level of serum phosphorus with no significant variation. The higher level of serum phosphorus in Group A might be due to supplementation of mineral mixture prior to heatsynch protocol. Phosphorus was associated with energy metabolism and its deficiency resulted in delayed sexual maturity, anoestrus, repeat breeding and irregular estrous cycle reported by Bindari (2013) [2]. Marginal deficiency of phosphorus was opined to be enough to cause disturbances in pituitary- ovarian axis without manifesting specific systemic deficiency symptoms (Buhecha, 2015) [3].

Level of serum zinc

The mean level of serum zinc in postpartum anoestrous crossbred cows on day 0, 7, 8 and 9 of treatment protocol in Group A was 107.69 ± 0.49, 108.31 ± 0.48, 108.39 ± 0.48 and 109.07 ± 0.47 (µg/dl) and was found to be higher than the Group B animals with no significant variation. Mohapatra *et al.* (2012) [5], Jana *et al.* (2015) [4] and Sahoo *et al.* (2016) [6] also reported significant increase in plasma zinc concentration after mineral supplementation. Zinc is essential for enzymatic reactions such as carbohydrate and protein metabolism, nucleic acid metabolism, vitamin A and E transport and utilization. Zinc is essential for proper sexual maturity, reproductive capacity and onset of estrus. Zinc also increases plasma beta carotene level which is directly correlated to improve conception rate and embryonic development (Bindari *et al.* 2013) [2].

Table 2: Estimation of blood bio hemicals in anoestrous rossbred cows on different days of with (Group A) and without treatment (Group A)

	Groups	Days of treatment				Significance	
		0 Day	7 Day	8 Day	9 Day	F Test	T test
Serum Oestrogen (pg/ml)	A	2.33A ± 0.07	5.34aB ± 0.11	8.28aC ± 0.16	18.33aD ± 0.30	730.528**	NS
	B	2.29 ± 0.06	2.36b ± 0.07	2.37b ± 0.05	2.39c ± 0.06	NS	
serum progesterone (ng/ml)	A	0.92A ± 0.02	2.69aB ± 0.01	0.65aC ± 0.01	0.39aD ± 0.01	286.147**	NS
	B	0.88 ± 0.04	0.90b ±0.01	0.89b ± 0.02	0.88b ± 0.02	NS	
serum cortisol (ng/ml)	A	6.20aA ± 0.12	6.29aB± 0.05	6.53aC ± 0.12	6.78aD ± 0.07	793.606**	NS
	B	5.16b ± 0.05	5.21b ± 0.06	5.22b ± 0.11	5.24b ± 0.05	NS	
serum cholesterol (mg/dl)	A	119.96b ± 0.67	119.65b ± 0.65	119.47b ± 0.69	119.31b ± 0.69	NS	39.671**
	B	111.91c ± 0.93	112.23c ± 1.04	112.50c ± 0.96	112.85c ±1.05	NS	
total serum protein (g/dl)	A	7.85b ± 0.07	7.89b ± 0.08	7.94b ± 0.10	8.00b ± 0.13	NS	NS
	B	6.88a ±0.14	6.89a ±0.14	6.92a ± 0.13	6.91a ± 0.14	NS	
serum calcium (mg/dl)	A	8.60a ± 0.02	8.63a ± 0.03	8.65a ± 0.03	8.66a ±0.03	NS	NS
	B	8.16b ±0.06	8.15b ± 0.06	8.17b ± 0.05	8.17b ±0.05	NS	
serum phosphorus (mg/dl)	A	4.16a ± 0.03	4.19a ± 0.02	4.21a ± 0.02	4.22a ± 0.03	NS	NS
	B	3.99b ±0.02	3.98b ±0.03	3.95b ±0.03	3.98b ±0.02	NS	
serum zinc (µg/dl)	A	4.16a ± 0.03	4.19a ± 0.02	4.21a ± 0.02	4.22a ± 0.03	NS	
	B	3.99b ±0.02	3.98b ±0.03	3.95b ±0.03	3.98b ±0.02	NS	

**, Significant at 0.01 level of probability, NS Non-significant

Conclusion

From the present investigation it could be concluded that the Heatsynch protocol supplemented with mineral mixture and bypass fat resulted in a high oestrus response with maximum conception rate at induced oestrus. It could be effectively used to overcome postpartum anoestrus in crossbred cows with better conception rate in comparison with heatsynch alone or heatsynch with mineral mixture supplementation. However, systematic studies on a large sample of cows are warranted before drawing concrete conclusions.

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