EFFECT OF HORMONAL PROTOCOLS ON HAEMATO-BIOCHEMICAL ESTIMATION IN POST PARTUM ANOESTROUS BUFFALOES DURING BREEDING SEASON

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ABSTRACT

The experiment was carried out in fourty post partum anoestrus buffaloes during breeding season. All selected animals were classified in 4 equal groups. Each group comprises 10 buffaloes. Group I was administered Ovsynch protocol with Progesterone impregnated intravaginal device which removed on 7th day and GnRH @ 10 mcg i/m on day of AI. Group II was advised Progesterone impregnated intra-vaginal device which removed on 7th day and GnRH @ 10 mcg i/m on day of AI. Group III was given Ovsynch protocol alone and Group IV was kept as control. Fixed timed Artificial Insemination was performed in I, II and III group of buffaloes. Blood was collected before and after experiment for estimation of haemato-biochemical parameters. The average values of WBC, RBC and Haemoglobin was observed non significant on 7th and 10th day. The level of total protein did not significantly changed but total cholesterol and glucose significantly ($P<0.01$) increased on day 0, 7th and 10th. There were no significant effects of the level of serum calcium and phosphorous on day 7th and 10th. The level of Cu, Fe and Zn increased significantly ($P<0.05$) on day 7th and 10th whereas Cu, Fe and Zn found significantly ($P<0.05$) greater on day 0, 7th and 10th. It can be concluded that effect of hormonal protocols is very effective for oestrous induction and conception rate in post partum anoestrous buffaloes during breeding season. However, significant difference in values of haemato-biochemical parameters was observed between pregnant and non pregnant animals whereas macro minerals and micro minerals did not altered.

***Key words*:** Hormonal protocol, haemato-biochemical estimation, anoestrus, buffaloes

Introduction

Buffalo is a multi-purpose species and contributes significantly to rural economy and dairy industry in many developing countries across the world. This species is also a most valuable livestock resource in Asian countries including India. Although buffaloes are well adapted to hot and humid climate, their reproductive performance is greatly influence by heat stress (Marai and Haeeb, 2010). Postpartum anestrus in buffaloes is one of most common reproductive disorder and higher incidences are recorded during summer season.

The resumption of oestrus post partum is a critical factor to achieve a satisfactory production in buffaloes. In dairy cattle, this period should be in about 60-80 days and conception must be obtained by 85-100 days post-calving to get desirable benefits. Unfortunately, postpartum estrus in buffaloes always comes much later than the normal figures. The long postpartum anestrus in the buffalo depends on several factors including: season of calving, uterine involution, suckling, milk yield, nutrition and body condition score at calving.

Buffalo suffers from several inherent reproductive problems such as seasonality of breeding, delayed puberty and sexual maturity, repeat breeding, prolonged post-partum anestrus, summer anestrus, and silent estrus (Nanda *et al.*, 2003; Das and Khan, 2010).

The energy requirements of lactating buffaloes also increased under high temperature conditions, but this increase seemed to be caused mainly by the increase in metabolic energy. Low fiber, high fermentable carbohydrate diets lower dietary heat increment compared with higher fiber diets. Although the metabolic energy of dairy buffaloes increases in a hot environment, heat stress depresses feed intake.

Excessive protein, especially soluble or degradable protein, has been suggested to decrease fertility of cows, possibly though early embryonic death. Cows make use of non-protein nitrogen in the rumen to make microbial protein, which is very high quality. The rumen microbes combine non-protein nitrogen with carbohydrate skeletons, such as volatile fatty acids, to make protein. Decrease feed intake can be determined by loss of body condition, poor growth rates, or decreased intake relative to National Research Council (NRC) requirements for beef or dairy cattle. Serum non-esterified fatty acids or ß-OH butyrate have been suggested as indicators of inadequate feed intake/negative energy balance in dry cows and early lactation cows.

Therefore, the present research study was conducted to observe the effect of hormonal protocols in haemato-biochemical on post partum anoestrus buffaloes during breeding season.

Materials and Methods

Total fourty post partum anoestrous buffaloes were selected for the present study. The experiment was carried

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out during breeding season. All animals were included in the experiment study on the basis of ovarian activity, body score condition and health status. Buffaloes were administered anthehelmentic treatment and also advised mineral mixture @ 50 gm/day/animal.

Group I was administered Ovsynch protocol (GnRH on day '0', PG on day '7' and GnRH on '9') + Progesterone impregnated intra-vaginal device which removed on 7th day. Group II was administered with Progesterone impregnated intra-vaginal device which removed on 7th day and GnRH @ 10 mcg i/m on day of AI. Group III was treated with Ovsynch protocol and Group VI was control.

Oestrous was detected and fixed timed Artificial insemination was done. Blood samples were collected from jugular vein of all selected buffaloes in heparinized vacutainers at day 0, 7th and 10th from all groups. Floride vacutainers were used for glucose estimation. The blood samples were centrifuged at 3000 rpm for 15 minutes and the serum was separated immediately. Serum was transferred to 2 ml storage vials and stored in properly labeled sterilized vials at -20°C until analysis.

The estimation of haematological parameters were attempted immediately after collection of the blood samples. Hemo-analyser (Model ABX Micros ESV60, HORIBA Medical, Japan) was used for the estimation of total WBC, RBC, Hb, MCV, MCH and MCHC. Estimation of serum total glucose, total Cholesterol, total protein, serum calcium and serum phosphorus was attempted with semi-automated biochemical analyser (*Seac STAR-21, Rapid Diagnostics Pvt. Ltd. Model no. S/N 322437*) and Cu, Fe, Zn, Co and Mn values were attempted with Atomic Absorption Spectrophotometer (Lab India). The data were analyzed statistically (Panse and Sukhatme, 1954).

Results and Discussion

The haemato-biochemical observations are given in Table 1.

In the present study, it was observed that overall value of WBC did not increased significantly in treatment and control groups during breeding season. The present findings are agreement with Kumar *et al.* (2014) who reported WBC count as 9.831 ± 0.404 vs 8.413 ± 0.599 $\times 10^6$/ml in postpartum anoestrus and normal cyclic Murrah buffaloes. Normal values of WBC ($x10^3$/µL) count in buffaloes ranges between 5.9 - 14.0 and present values are well within the range.

On day 0, the overall RBC values did not vary significantly of all groups, in present experiment. But on day 7th and 10th, the overall values of RBC was increased significantly (P<0.01) in Group I than II, III and IV. Kumar *et al.* (2014) who reported mean RBC ($\times 10^9$/ml) as 7.406 ± 0.337 vs 6.357 ± 0.5460 in postpartum anoestrus and normal cyclic Murrah buffaloes.

On day 0, the Hb values did not significant in all groups. On day 7th and 10th, in Group I Hb values were found to be increased significantly (P<0.01) than Group II, III and IV in the present study. Gyan *et al.* (2017) who reported as no significant difference recorded in Hb, TLC, DLC value among non-cyclic and cyclic Murrah buffaloes. Hedaoo *et al.* (2008) noted 12.96 ±0.37 and 9.98 ±0.30 g% in cyclic and non cyclic buffaloes.

Overall MCV values of Group I, II significantly (P<0.01) increased than group III and IV. Aggarwal *et al.* (2016) reported 46.83±1.44 to 48.63±1.01 fl and Ghani *et al.* (2017) reported 64.21±3.55 vs 56.81±5.35fl in cyclic and acyclic buffaloes.

The MCH values of treatment groups was significantly (P<0.1) greater than the control group. Ghani *et al.* (2017) reported 21.58±5.47 vs 15.99±1.84 pg in cyclic and acyclic buffaloes. Similar findings with other blood constituents were reported in the present study. As there is no significantly differed in overall MCHC values of all groups in buffaloes under research study. Kumar (2015a) who recorded the difference in values of MCV, MCH and MCHC on the day of treatment and on the day of oestrus were non-significant (P>0.05) with Ovsynch and Ovsynch + CIDR treatment whereas in the present study no significant changes found in values of the same.

On day 0, overall serum glucose level of all groups was observed in normal range. On day 7th and 10th, serum glucose level was found significantly (P<0.01) increased in Group I and III than Group II and IV. Blood glucose (energy), progesterone and fertility are directly proportionally to each other in animal reproduction and all reproduction events are expected only when blood glucose levels are very normal and optimum. Nutrition provides blood glucose and thus relates with reproduction.

Jayachandran *et al.* (2013) recorded glucose (mg/dl) as 51.86 ± 1.01, 56.90 ± 3.30 in anoestrous and regular cyclic values, respectively. Kumar *et al.* (2015b) noted glucose (mg/dl) as 72.25±4.11 and 68.4±5.03 in oestrum and anoestrum buffaloes, respectively.

Day 0, 7th and 10th, total protein level did not increased significantly between the all groups. It was found under normal range. Akhtar *et al.* (2010) investigated 8.39±0.55 and 7.47±0.88 g/dl between cycling versus anestrous Nili-Ravi buffaloes and Kumar *et al.* (2015b) reported 7.9±0.4, 6.94±0.19 g/dl in oestrum and anoestrum buffaloes.

On day 0, the overall cholesterol level was found under normal range in all groups. On day 7th, total cholesterol level was increased significantly (P<0.01) between treatment groups than control. On day 10th, there were no significant changes found in overall cholesterol level of all groups. Akhtar *et al.* (2010) investigated 155.58±7.15 and 133.82±8.12 g/dl between cycling versus anestrous Nili-Ravi buffaloes and Kumar *et al.* (2015b) reported 101.68±7.8 and 123.4±13.37 mg/dl in estrum and anestrum buffaloes. Whereas, Kumar *et al*. (2015a) observed the mean cholesterol level non-significantly (P>0.05) higher on the day of estrus as compared to the day of treatment in Ovsynch and Ovsynch + CIDR protocol.

Day 0, 7th and 10th, overall serum calcium level did not increased significantly between the treatment and control group. Serum calcium is not directly related with estrus and cyclicity but has major role for smooth parturitions. However, role of serum calcium can not be ignored as ratio of Ca:P affects cyclicity in bovines. Jayachandran *et al.* (2013) found as 8.63 ± 0.25 and 8.32 ± 0.36 mg/dl in anoestrus and regular cyclic buffaloes. Kumar *et al*. (2015a) recorded as 7.18±0.14, 7.66±0.19 mg/dl on day of treatment and day of oestrus in buffaloes, respectively.

Day 0, 7th and 10th, overall serum phosphorous level did not increased significantly between the treatment and control group in buffaloes. Kumar *et al.* (2015a) recorded as 3.87±0.16 and 4.33±0.24mg/dl on day of treatment and day of oestrus in buffaloes.

Table 1: Efficacy of various hormonal protocols on oestrus induction and conception rate in post partum anoestrous buffaloes during breeding season.

Parameters	0 day	7 day	10 day	CD (5%/**1%**)
WBC	8.61±0.13	9.15±0.10	9.65±0.12	NS
RBC	7.18±0.21	7.81±0.20	8.17±0.27	NS
HGB	10.56±0.22	11.07±0.44	11.67±0.34	NS
MCV	51.88±1.38*	52.52±1.53*	52.68±1.65*	NS
MCH	17.16±0.31*	18.05±0.53*	18.17±0.62*	NS
MCHC	33.25±0.24*	34.22±0.34*	34.44±0.15*	NS
Glucose	53.50b±0.17*	54.95a±0.588	56.61a±1.07*	1.45/1.75
Protein	7.03±0.13	6.98±0.08	7.00±0.09	NS
Cholesterol	112.83c±3.84**	120.17b±3.55**	126.99a±2.86**	4.37/5.92
Ca	9.03±0.08	9.56±0.10	9.44±0.22	NS
P	5.19±0.11	5.31±0.12	5.36±0.12	NS
Cu	0.75c±0.03	0.84b±0.03*	0.89a±0.04*	0.08
Fe	1.59b±0.06*	1.70a±0.08*	1.79a±0.09*	0.10
Zn	1.72b±0.06*	1.90a±0.10*	2.01a±0.15*	0.12
Co	0.48±0.06	0.50±0.03	0.52±0.03	NS
Mn	0.39±0.02	0.44±0.01	0.46±0.01	NS

Note: Superscripts of small alphabets indicates significance (P<0.01) between days; * Indicates significance between breeding and non breeding season (P<0.05). **Indicates significant difference between breeding and non breeding season (P<0.01)

Table 1: Efficacy of various hormonal protocols on oestrus induction and conception rate in post partum anoestrous buffaloes

Group	I			II			III			IV		
Parameters	0 day	7 day	10 day	0 day	7 day	10 day	0 day	7 day	10 day	0 day	7 day	10 day
WBC	8.70 ±0.37	9.29 ±0.38	9.89 ±0.45	8.34 ±0.23	9.33 ±0.35	9.43 ±0.32	8.46 ±0.18	9.03 ±0.27	9.47 ±0.33	8.93 ±0.25	8.93 ±0.25	9.81 ±0.37
RBC	7.13 ±0.41^{b}	8.25 ±0.41^{ab}	8.68 ±0.41^{a}	6.96 ±0.45	7.39 ±0.32	7.59 ±0.32	6.84 ±0.28	7.58 ±0.30	7.85 ±0.29	7.78 ±0.26	8.01 ±0.25	8.57 ±0.35
HGB	10.95 ±0.40^{b}	11.63^{A} ±0.31^{ab}	12.26 ±0.26^{a}	10.93 ±0.62^{a}	11.94^{A} ±0.64^{b}	12.21 ±0.69^{b}	10.33 ±0.30^{a}	10.73^{AB} ±0.35^{b}	11.39 ±0.41^{b}	10.04 ±0.35	9.99^{B} ±0.32	10.83 ±0.33
MCV	55.00^{A} ±1.28	55.95^{A} ±1.18	56.46^{A} ±1.10	53.42^{A} ±1.80	54.19^{A} ±1.85	54.31^{A} ±1.86	49.49^{B} ±0.52	50.51^{B} ±0.55	50.71^{B} ±0.59	49.61^{B} ±0.73	49.43^{B} ±0.65	49.23^{B} ±0.81
MCH	17.85 ±0.50	19.04^{A} ±0.48	19.48^{A} ±0.48	17.43 ±0.46	18.50^{A} ±0.68	18.61^{A} ±0.66	16.94 ±0.37	18.07^{A} ±0.45	18.06^{B} ±0.46	16.44 ±0.33	16.57^{B} ±0.28	16.52^{C} ±0.26
MCHC	32.56 ±0.53	33.73 ±0.45	34.09 ±0.55	33.27 ±1.22	34.36 ±1.12	34.40 ±1.14	33.59 ±0.99	35.12 ±0.75	34.81 ±0.86	33.59 ±0.72	33.67 ±0.62	34.45 ±0.73
Glucose	53.51 ±0.98^{b}	56.18 ±1.09^{a}	58.43 ±0.94^{a}	53.96 ±1.66	55.30 ±1.42	56.11 ±1.28	53.13 ±0.79^{b}	54.90 ±0.69^{b}	58.11 ±1.07^{a}	53.42 ±1.48	53.41 ±1.10	53.80 ±1.12
Protein	6.87 ±0.14	7.12 ±0.15	7.11 ±0.13	7.42 ±0.11	6.86 ±0.20	7.01 ±0.14	6.88 ±0.09	7.11 ±0.16	7.14 ±0.09	6.95 ±0.16	6.83 ±0.13	6.76 ±0.22
Cholesterol	118.36 ±5.33	125.31^{A} ±4.18	130.30 ±4.20	120.22 ±4.12	125.55^{A} ±3.69	132.86 ±3.31	108.36 ±3.31	110.42^{B} ±4.31	124.71 ±13.38	113.48 ±4.69	119.40^{B} ±5.40	120.08 ±5.86
Ca	9.26 ±0.28	9.83 ±0.31	9.87 ±0.25	9.00 ±0.17	9.44 ±0.20	9.34 ±0.15	9.00 ±0.19	9.56 ±0.24	9.68 ±0.28	8.88 ±0.28	9.39 ±0.24	8.88 ±0.37
P	4.86 ±0.14	5.40 ±0.17	5.37 ±0.20	5.25 ±0.16	5.23 ±0.10	5.31 ±0.35	5.38 ±0.20	5.58 ±0.25	5.67 ±0.23	5.25 ±0.12	5.04 ±0.13	5.11 ±0.12
Cu	0.82 ±0.02^{b}	0.93^{A} ±0.04^{ab}	1.00 ±0.06^{a}	0.72 ±0.03	0.79^{B} ±0.04	0.84 ±0.04	0.70 ±0.03^{b}	0.80^{B} ±0.03^{a}	0.87 ±0.04^{a}	0.78 ±0.03	0.82^{B} ±0.04	0.86 ±0.03
Fe	1.76 ±0.12^{c}	1.91 ±0.09^{b}	2.04 ±0.06^{a}	1.55 ±0.12	1.72 ±0.13	1.77 ±0.15	1.53 ±0.10	1.64 ±0.12	1.74 ±0.15	1.52 ±0.09	1.52 ±0.11	1.60 ±0.08
Zn	1.73 ±0.15^{b}	2.17 ±0.21^{a}	2.45^{A} ±0.24^{a}	1.56 ±0.12	1.71 ±0.09	1.78^{B} ±0.10	1.75 ±0.17	1.83 ±0.16	1.91^{B} ±0.16	1.84 ±0.03	1.88 ±0.05	1.91^{B} ±0.0
Co	0.36 ±0.01	0.42 ±0.01	0.45 ±0.01	0.41 ±0.02	0.48 ±0.05	0.52 ±0.06	0.58 ±0.04	0.55 ±0.03	0.57 ±0.03	0.58 ±0.03	0.56 ±0.03	0.57 ±0.03
Mn	0.34 ±0.03	0.41 ±0.05	0.42 ±0.05	0.39 ±0.02	0.45 ±0.02	0.48 ±0.02	0.41 ±0.03	0.47 ±0.03	0.49 ±0.02	0.43 ±0.03	0.44 ±0.02	0.46 ±0.02

Note: Superscripts small alphabets indicates significant between the days and Capital alphabets indicate significance between the groups

On day 0, the overall Cu level was no significant differed in all groups. On day 7th and 10th, in group I Cu level was increased significantly (P<0.01) than Group II, III and IV. There was no significant difference of overall Fe, Zn, Co and Mn level during the study in all groups. Kumar *et al.* (2015c) recorded Zn level as 1.0 ± 0.1in cyclic buffalo during summer season.

It was concluded that no significant difference between treatment and control group was observed in haemato-biochemical parameters. Macro minerals such as calcium and phosphorous and micro minerals such as Cu, Fe, Zn, Co and Mn values did not altered with the administration of hormonal protocols in post partum anoestrus buffaloes during breeding seasons.

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