



## Effect of Heat Stress on Haemato-biochemical and Endocrinological Profile

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### ABSTRACT

Present study was planned to investigate the cause of postpartum true anoestrus in buffaloes during summer through the analysis of hemato-biochemical and endocrinological profile of anoestrus buffaloes. Blood samples were collected from 50 buffaloes belonging to two groups i.e. cyclic (n=10), and summer anoestrus buffaloes (n=40) animals respectively. The samples were analysed for haematological parameters including total erythrocytes count (TEC), total leukocytes count (TLC), haemoglobin (Hb), packed cell volume (PCV), erythrocyte sedimentation rate(ESR), mean corpuscular haemoglobin concentration(MCHC), biochemical parameters viz., glucose, total protein, albumin, total cholesterol, urea, creatinine, alkaline phosphatase, alanine amino transaminase and aspartate transaminase, calcium, phosphorus, manganese, zinc, copper cobalt and hormones of the plasma sample viz., progesterone, estradiol tri-iodothyronine (T3) thyroxine (T4) and cortisol using commercially available kits. The results revealed that Hb and MCH varied significantly ( $P<0.05$ ) between the groups and other haematological parameters did not vary between two groups. In biochemical parameters glucose, total protein, albumin and total cholesterol were significantly ( $p<0.05$ ) lower in anoestrus buffaloes than normal cyclic buffaloes and the progesterone, estradiol, tri-iodothyronine and the cortisol concentration varied significantly ( $P<0.05$ ) between two groups. It can be concluded that variation in some hemato-biochemical and hormonal levels might be the cause of the anoestrus during summer stress in buffaloes.

**Keywords:** Summer Anoestrus buffaloes, Hematological, Biochemical and Endocrinological profile.

Buffalo (*Bubalus bubalis*) is known as the world's second most important milch animal because it shares more than 95% of the milk produced in South Asia (Javaid *et al.* 2009). Anoestrus is one of the most commonly occurring reproductive problems in buffaloes in India, affecting livestock productivity and economics to a greater extent. This problem is more severe in sub urban and rural areas of the country. It is a functional irregularity of the reproductive cycle which is characterised by the absence of overt signs of oestrus manifested either due to lack of expression of oestrus or failure of its deduction. Anoestrus is observed in post pubertal heifer buffaloes. It is also observed in adult buffaloes during pregnancy, lactation, and early postpartum period during summer. Incidence of anoestrus though varies in the different managerial system but it is more common in buffaloes than cattle and especially during summer. (Kumar *et al.* 2014)

Heat stress has a direct effect on breeding efficiency of

female buffaloes and reduces the intensity and duration of oestrus and causing huge economic losses to the buffalo breeders as well as dairy industry. Anoestrus in buffaloes during summer is due to lower circulating concentration of hypophyseal and gonadal hormones and suboptimal functioning of hypothalamo hypophyseal and gonadal axis. The post-partum cyclic activity depends on the synchronous activity of hypothalamic –pituitary-ovarian axis (Perea and Inskeep 2008).

Objectives of this study is to investigate the haematological, biochemical and various hormonal profile in anoestrus buffaloes during summer under field conditions to determine the etiology of the problems so that appropriate treatment might be given to the animals by using mineral mixture with some artificial progesterone device in order to improve the reproductive efficiency. The knowledge of its incidence may be helpful to adopt therapeutic measures for the benefit of dairy farmers.

## MATERIALS AND METHODS

The study was carried out on 50 non-descriptive rural buffaloes belonging to villages around Tirunelveli district of Tamil Nadu during summer (March-June) with mean ambient temperature and relative humidity ranging from 38-43°C and 50-60% respectively.

Out of 50 animals 10 animals were normal cyclic and 40 were post partum anoestrus for more than 5 months after calving. Before the start of the experiment, the animals were examined per rectum twice at 10 days interval to confirm ovarian activity. Blood samples (approximately 10 ml) were collected aseptically from the jugular vein of each animal in vacutainer tubes with anticoagulant using disodium ethylene diamine tetra acetic acid (EDTA). Two ml of this blood was poured in a separate vial for haematological studies. In the remaining amount of plasma was separated by centrifugation at 3000 rpm for 15 minutes and transferred into sterile tubes, labelled and stored at -20°C until analysed for biochemical and hormonal parameters.

Blood samples were analysed for haematological parameters viz., haemoglobin (Hb), red blood cells (RBC), hematocrit (PCV), white blood cells (WBC), different leukocytes, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined manually and the plasma biochemical parameters viz., glucose, total protein, albumin, total cholesterol, urea, creatinine, alkaline phosphatase, alanine transaminase and aspartate transaminase were analysed by using Photo electric colorimeter. The plasma mineral, calcium, phosphorus, manganese, zinc, copper and cobalt were determined by atomic spectrophotometer.

Hormones of the plasma sample viz., Progesterone, Estradiol, Tri-iodothyronine (T3) Thyroxine (T4) and Cortisol, were analysed by using RIA kits purchased from Anand Brothers, Ahmedabad and the estimation was carried out at National Institute of Animal Nutrition and Physiology (NIANP), Bangalore.

Statistical data were analysed by the descriptive statistical tool (computer software Microsoft Excel 2010, beta) and expressed at 5% level of confidence as mean and standard error.

## RESULTS AND DISCUSSION

The normal cyclic animal significantly ( $p < 0.05$ ) had higher concentration of haemoglobin than summer anoestrus buffaloes (Table 1) The lower concentration of haemoglobin (g %) in summer anoestrus concurred with Kumar *et al.* (2014) who reported that the haemoglobin value of post partum anoestrus buffaloes was lower when compared with normal cyclic buffaloes during low breeding season.

A decrease in Hb value is indicative of certain systemic disorders which could indirectly affect the functional activity of the reproductive organs. However, the other haematological parameters did not differ significantly between normal cyclic and summer anoestrus buffaloes.

The plasma glucose level in normal cyclic buffaloes was significantly ( $p < 0.05$ ) higher when compared to anoestrus buffaloes during summer months (table 2). The lower level of glucose in the present study was in agreement with findings of Shaffer *et al.* (1981) who identified a highly significant effect of seasonal temperature on blood glucose levels. The lower level of serum glucose in summer anoestrus buffaloes might be due to hypothalamic failure in utilizing the glucose or may be due to lower energy intake (McClure, 1965).

The normal cyclic animals had a significantly ( $P < 0.05$ ) higher concentration of total protein and albumin as compared with the anoestrus buffaloes. Which were similar to the results of Shafie and Badreldin (1962) who reported that the total protein in buffaloes exposed to direct solar radiation in Egypt was decreased by 11.9%.

The total cholesterol values (mg/dl) of normal cyclic buffaloes were higher than the summer anoestrus buffaloes. The total cholesterol level in the normal cyclic buffaloes were found to be significantly ( $P < 0.05$ ) higher than the summer anoestrus buffaloes. It might be due to non-utilization of cholesterol for steroid hormone synthesis. There is no significant difference noticed in all other biochemical parameters between normal cyclic and summer anoestrus buffaloes.

The mean progesterone (ng/ml), estradiol (pg/dl) and tri-iodothyronine (ng/ml) concentration differed significantly ( $P < 0.05$ ) between normal cyclic and summer anoestrus buffaloes. There was a significant ( $P < 0.05$ ) reduction in all three hormones in summer anoestrus buffaloes

(table 3). The plasma progesterone concentration in the present study was significantly ( $p<0.05$ ) lower in summer anoestrus buffaloes than normal cyclic buffaloes. Ullah *et al.* (2006) reported that the true anoestrus buffaloes had  $<0.25$  ng/ml of progesterone in their plasma. The estradiol concentration was significantly ( $p<0.05$ ) higher in normal cyclic buffaloes than summer anoestrus buffaloes. The lower level of estradiol concentration in summer anoestrus buffaloes in the present study concurred with the study of Rao and Pandey, 1983 reported that plasma estradiol concentrations were found to be lower in summer compared to cooler months.

The plasma concentration of tri-iodothyronine in the present study was significantly ( $p<0.05$ ) higher in normal cyclic buffaloes when compared with summer anoestrus buffaloes.

The lower level of tri iodothyronine observed in summer anoestrus buffaloes in this study concurred with the Marai and Haebe (2010) who suggest that the decrease in tri iodothyronine in response to heat stress can act as an adaptive mechanism to reduce heat production. Conversely Aggarwal and Singh (2010) reported that there was no change in tri iodothyronine concentration in two cooling systems (sprinkling and immersion) and two period of year (hot-dry and hot-humid) in lactating buffaloes.

There was no significant difference in thyroxine concentration in this study, which agreed with the findings of Sharma *et al.* (1998) who reported that there was no significant difference between T3 and T4 concentration in cyclic and anoestrus buffaloes but Dutta *et al.* (1990) and Sarvaija (1991) reported low T3 and T4 profiles in anoestrus buffaloes and cow heifers respectively. Mudgal (1992) reported that hypothyroid condition reduces the responsiveness of ovary to pituitary gonadotropins that reduces the thyroid level and may cause anoestrus in buffaloes.

The plasma cortisol concentration was significantly ( $p<0.05$ ) higher in summer anoestrus buffaloes than compared with normal cyclic buffaloes recorded in the present study concurred with the findings of Marai and Haebe (2010) who reported that the concentration of cortisol increased under heat stress in anoestrus buffaloes during summer.

**Table 1.** Comparative haematological parameters of normal cyclic and Summer anoestrus buffaloes

Variables	Normal cyclic buffaloes (n=10)	Anoestrus buffaloes (n=30)
Hb (gm/dl)	12.00±3.75 <sup>a</sup>	11.77±2.05 <sup>b</sup>
PCV (%)	35.20 ±1.03	35.53± 1.06
RBC ( $\times 10^6/\text{mm}^3$ )	6.91 ±2.11	7.06± 0.19
WBC ( $\times 10^3/\text{mm}^3$ )	7.64± 0.26	7.92 ± 0.91
MCV (fl)	50.57± 1.24	50.68±1.43
MCH (pg)	17.24±0.41 <sup>a</sup>	16.49±0.48 <sup>b</sup>
MCHC (%)	34.09± 1.32	33.12±1.67
Neutrophils (%)	30 ±1.58	36± 2.92
Lymphocytes (%)	56.7± 1.49	56.02 ±1.37
Eosinophils (%)	7.2± 1.31	7.25 ±1.05*
Monocytes (%)	5.0 ±0.82	4.63± 0.63
Basophils (%)	0.24 ±0.05	0.24± 0.5

Means within the same row bearing different superscripts differ significantly  $P<0.05$

**Table 2.** Comparative Plasma profile of biochemical parameters of normal cyclic and summer anoestrus buffaloes

Variables	Normal cyclic buffaloes(n=10)	Anoestrus buffaloes(n=30)
Glucose (g/dl)	36.8 ± 0.63 <sup>b</sup>	35.57± 1.69 <sup>a</sup>
Total protein (g/dl)	7.9 ± 0.35 <sup>b</sup>	6.46± 0.44 <sup>a</sup>
Albumin (g/dl)	4.47±0.51 <sup>b</sup>	3.25± 0.26 <sup>a</sup>
Total cholesterol (mg/dl)	123.0±1.79 <sup>b</sup>	107.27± 3.32 <sup>a</sup>
Urea (mg/dl)	11.93± 0.22	11.87± 0.27
Creatinine (mg/dl)	1.4 ± 0.09	1.37 ± 0.14
AST (U/l)	102.17 ±0.88	102.78 ± 0.97
ALP (U/l)	87.7 ±1.83	87.55 ± 1.57
ALT (U/l)	25.2 ±1.03	25.85 ±1.23
Calcium (g/dl)	8.42 ± 0.23	8.47 ± 0.11
Phosphorus (g/dl)	3.68 ± 0.11	3.31± 0.15
Manganese ( $\mu\text{g/dl}$ )	0.169 ±0.004	0.154 ± 0.016



Zinc (µg/dl)	1.140 ± 0.014	1.100 ± 0.021
Copper (µg/dl)	0.661 ± 0.022	0.644 ± 0.039
Cobalt (µg/dl)	0.298 ± 0.018	0.260 ± 0.021

Means within the same row bearing different superscripts differ significantly P<0.05

**Table 3.** Comparative plasma profile of Progesterone, Estradiol, Triiodothyronine (T<sub>3</sub>), Thyroxine (T<sub>4</sub>) and Cortisol in normal cyclic and anoestrous buffaloes

Plasma Constituents	Normal cyclic buffaloes(n=10)	Anoestrous buffaloes(n=30)
Progesterone	1.81 ± 0.03 <sup>b</sup>	0.38 ± 0.02 <sup>a</sup>
Estradiol	17.95 ± 0.18 <sup>b</sup>	13.18 ± 0.18 <sup>a</sup>
Tri-iodothyronine	2.119 ± 0.059 <sup>b</sup>	1.742 ± 0.059 <sup>a</sup>
Thyroxine	53.072 ± 0.572	53.298 ± 0.572
Cortisol	1.568 ± 0.075 <sup>a</sup>	2.564 ± 0.07 <sup>b</sup>

Means within the same row bearing different superscripts differ significantly P<0.05)

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