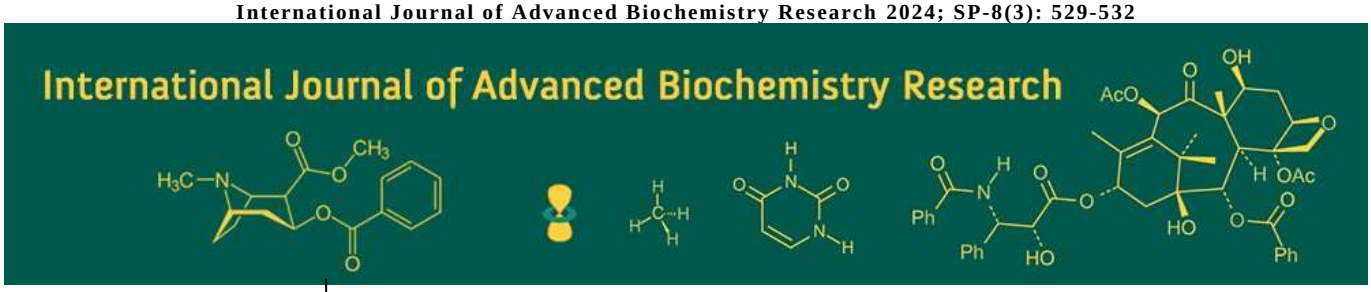

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Blood glucose concentrations in anestrus Murrah buffalo and heifer during summer and winter season

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Abstract
During immediate post-partum period, metabolic changes induced by mismatching of energy requirements and energy intake may lead to negative energy balance, which impacts the subsequent fertility. The blood biochemical profiles are considered important in evaluating the health status of animals. Blood glucose level is an important biochemical constituent required for normal reproductive performance of buffalo. So, the study was designed to investigate the level of plasma glucose level anoestrous buffaloes and buffalo heifers. Study done on 193 anoestrous buffaloes and buffalo heifers and concluded that blood glucose concentrations remain similar in all post-partum anoestrous Murrah buffalo and heifer during summer and winter seasons.

Keywords: Buffalo, heifer, glucose, anoestrous, farm, field, summer, winter

Introduction
Buffalo is one of the most important dairy animal which being reared mainly in tropical and sub-tropical countries. In India, it contributes more than 55 percent of the total milk production. In addition, buffalo also plays a significant role as draught and meat animal hence it have an important role in national economy. Livestock sector contributes 4.11% GDP and 25.6% of total Agriculture GDP to the nation. Apart from a good producer, the buffalos can utilize poorer quality roughages, adapt to harsher environments and are more resistant to several bovine tropical diseases. In queue of being topper, India also attained first position in world for its highest milk production during year 2016-17 of which more than 55% of this milk production is contributed by buffaloes. India is privileged to have the home-tract of Murrah buffalo where this species also known as "Black Gold" because of its inherent capacity to produce plenty of milk.

Low reproductive efficiency in the buffalo remains a major economic problem globally due to inherent reproductive problems including delayed sexual maturity, seasonality in breeding, anestrus, long calving interval, silent heat coupled with poor expression of estrus, low conception rate and high thermal and lactation stress (Nanda *et al.* 2003, Terzano *et al.* 2012) [13, 19]. During immediate post-partum period, metabolic changes induced by mismatching of energy requirements and energy intake may lead to negative energy balance, which impacts the subsequent fertility. Assessing metabolic profile of dairy animals can reveal the reasons behind differential fertility (Jorritsma *et al.* 2003) [7]. Profile of blood metabolites have been used widely to identify problem and to indicate dietary causes of diseases or low production (Lee *et al.*, 1978) [12]. The blood biochemical profiles are considered important in evaluating the health status of animals. Heat stress causes reduction in dry matter intake that leads to negative energy balance and hypoglycemia which leads to anovulation and anoestrous conditions in buffalo (Roche and Diskin 2001, Hala *et al.* 2009) [15, 5]. Because plasma glucose is a metabolic signal providing information for control of GnRH releases (Foster and Nagatani 1999) [4] and has an important role in ovarian activity. Blood glucose level is an important biochemical constituent required for normal reproductive performance of buffalo. So, the study was designed to investigate the level of plasma glucose level anoestrous buffaloes and buffalo heifers.

Materials and Methods

The present study was carried out on 193 animals including pluriparous Murrah buffaloes (n=141) and buffalo heifers (n=52) maintained at 1) Central Institute for Research on Buffalo, Hisar (n=88), and 2) in rural areas, nearby Hisar district (n=105) during summer (May to August) and winter (November to February). The selected animals had a history of anestrous without showing any proper signs of heat such as bellowing, micturition, restlessness, vaginal discharge for the past more than 60 days following calving in post-parturient buffaloes, and after attaining the age of sexual maturity in heifers. Under field conditions, all animals were subjected to repeated rectal examination at 12 days interval for confirmation of anoestrous condition and to confirm absence of any cyclic structure on either of the ovary. In farm animals, additionally transrectal sonography was also done to confirm acyclicity without any ovarian structure before the start of estrus induction protocols in farm condition. All animals included in the field study were reared on stall-feeding in their respective villages, and milked and suckled twice a day. The stall feeding practices were as per the availability of seasonal green fodder and wheat straw, with concentrates as per recommended the production potential of individual animals. The study was conducted in two experiments as per parity of animal and which was further categorized into different groups.

Experiment 1: The study was conducted on 143 anestrous healthy pluriparous buffalo's belonged (between 2nd and 5th parity) and categorized in different groups according to the days post-partum and location of buffalo as given below:

Group 1 (n= 16): Anoestrous buffaloes of 60 to 90 days post-partum reared under farm conditions during winter.

Group 2 (n= 17): Anoestrous buffaloes of 60 to 90 days post-partum reared under farm conditions during summer.

Group 3 (n= 17): Anoestrous buffaloes of>90 days post-partum reared under farm conditions during winter.

Group 4 (n= 17): Anoestrous buffaloes of >90 days post-partum reared under farm conditions during summer.

Group 5 (n= 10): Anoestrous buffaloes of 60 to 90 days post-partum reared under field conditions during winter.

Group 6 (n= 23): Anoestrous buffaloes of 60 to 90 days post-partum reared under field conditions during summer.

Group 7 (n= 14): Anoestrous buffaloes of >90 days post-partum reared under field conditions during winter.

Group 8 (n= 27): Anoestrous buffaloes of >90 days post-partum reared under field conditions during summer.

Experiment 2: The study was conducted under farm and field conditions involving 52 healthy anoestrous buffalo heifers who had attained age of sexual maturity and had not shown signs of estrus. The buffalo heifers were free from any infectious or anatomical reproductive problem and had normal genitalia. The criteria to confirm anoestrous was the same as followed for buffalo in experiment number 1. Their anoestrous condition was confirmed on the basis of history obtained from farmers and subsequently confirmed through rectal examination at 12 days interval. The study was conducted during summer (n=31) and winter (n=21) months to check the efficacy of estrus induction protocol described above in experiment 1. The buffalo heifers were divided in different group as mentioned below:

Group 9 (n= 11): Anoestrous buffalo heifers reared under farm conditions during winter

Group 10 (n= 10): Anoestrous buffalo heifers reared under farm conditions during summer.

Group 11 (n= 10): Anoestrous buffalo heifers reared under field conditions during winter.

Group 12 (n= 21): Anoestrous buffalo heifers reared under field conditions during summer

Blood was collected from jugular vein in heparinised vacutainer vials. Blood glucose level was estimated through Accu-Chek Active device (Model GU) on the day 0 to diagnose the energy level of the buffalo.

Procedure: Test strip removed out from Accu-Chek Active test stripcontainer. Closed the test strip container again immediately. Hold the test strip showing the arrows printed on it and the green square face in upward direction. Gently slide the test strip into the test strip guide in the direction of the arrows until you feel it lock into place. The test strip must lie flat on the measurement window cover. Put 1–2µLof fresh blood on the pointed area and let the strip inside of device for approximately 5 seconds. The label on the test strip container shows blood glucose values in mg/dL and mmol/L next to each coloured dot.

Measuring interval: 10–600 mg/dL (0.6–33.3 mmol/L)

Results and Discussion

In earlier studies, the blood glucose levels were considered as parameter of energy status of bovine, so, in order to confirm the energy status in present study, we estimated the blood glucose and are depicted in table 1 and figure 1. We found similar (P>0.05) blood glucose levels on the day of start of protocol among buffalo (60-90 days post-partum and >90 days post-partum) and heifer groups. During post-partum period, negative energy balance may cause anovulation which leads to physiological anestrous (Kumar *et al*., 2014) [11]. It is obvious for the most of the cows to remain in anestrous during 10 to 12 weeks post-partum due to state of negative energy balance and thereafter, animal may be cyclic again (Peter *et al*., 2009) [14]. The present study revealed similar blood glucose levels in each group, and further didn't differ between winter and summer season also. The present study didn't compare the blood glucose level in comparison to control animals, however, the blood glucose levels were observed higher than earlier studies reported in both cyclic and non-cyclic buffaloes (Jayachandran *et al*., 2013; Anita *et al*., 2004; Kumar *et al*., 2019) [6, 2, 10]. On the other hand, previous studies reported significant different blood glucose levels between cyclic and non-cyclic buffaloes (Singh *et al*., 2006; Kabir *et al*., 2001; Khasatiya *et al*., 2005; Bohara and Deokata, 2009 and Akhtar *et al*., 2010) [18, 8, 9, 3-1]. As per the present findings,

the higher blood glucose level in buffalo seems more than enough as compared to normal levels. In spite of higher blood glucose concentration in the current study, anestrus condition in buffalo indicated that blood glucose was not the only important regulator responsible for ovarian cyclicity. Nevertheless, the hypoglycemic state in buffaloes reduced the hypothalamic-hypophysial-ovarian axis signal transmission leading to anoestrus condition (Sharma *et al.*, 1998) [17].

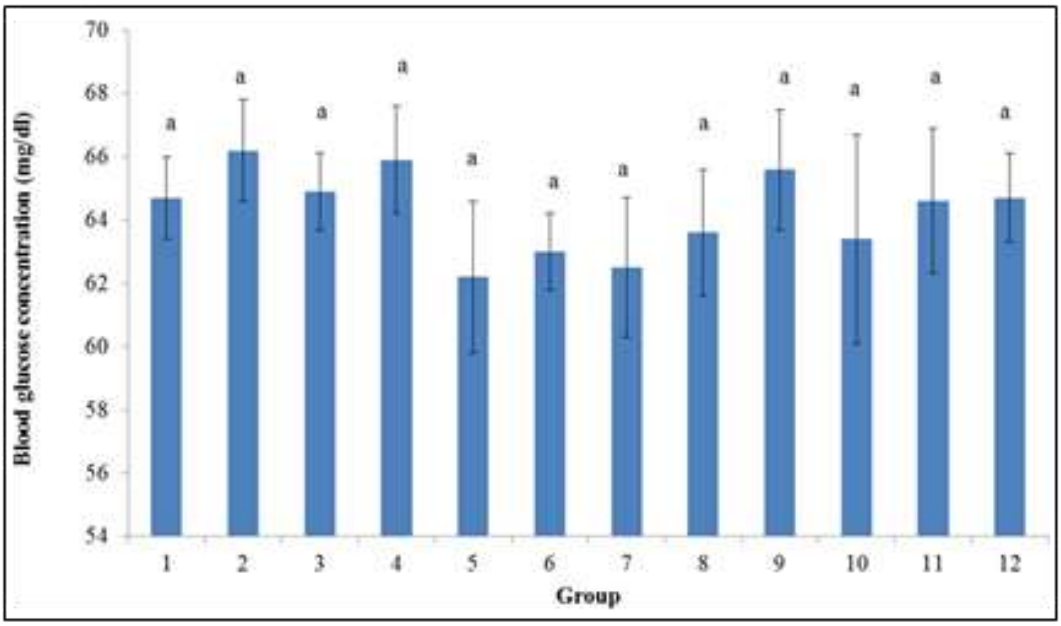

Fig 1: Blood glucose levels (mg/dl) of different groups of buffaloes and buffalo heifers on the day of start of estrous induction protocol. 'similar superscript do not differ significantly (P<0.05)

Table 1: Blood glucose levels (mg/dl) of buffaloes and buffalo heifers of different groups on the day of start of estrus synchronization protocol

Group	Season	Blood glucose
1 (n=16)	Winter	64.7 ± 1.3[a]
2 (n=17)	Summer	66.2 ± 1.6[a]
3 (n=17)	Winter	64.9 ± 1.2[a]
4 (n=17)	Summer	65.9 ± 1.7[a]
5 (n=10)	Winter	62.2 ± 2.4[a]
6 (n=23)	Summer	63.0 ± 1.2[a]
7 (n=14)	Winter	62.5 ± 2.2[a]
8 (n=27)	Summer	63.6 ± 2.0[a]
9 (n=11)	Winter	65.6 ± 1.9[a]
10 (n=10)	Summer	63.4 ± 3.3[a]
11 (n=10)	Winter	64.6 ± 2.3[a]
12 (n=21)	Summer	64.7 ± 1.4[a]

[a] similar superscript do not differ significantly (P<0.05)

Conclusion

The blood glucose concentrations remain similar in all post-partum Anestrus Murrah buffalo and heifer during summer and winter seasons.

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