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Assessment of Blood Metabolites during Early Postpartum Period as an Indicator of Reproductive Performance in Mehsana Buffaloes

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ABSTRACT

Background: The postpartum period being a crucial on future performance and fertility, it should be monitored critically and evaluated. Therefore, the study was designed to investigate associations between the metabolic indicators during early postpartum period with uterine diseases and the subsequent effect on reproductive performance.

Methods: The blood metabolites including non-esterified fatty acid (NEFA), beta hydroxy butyrate (BHBA), cholesterol, total protein, blood glucose and calcium are studied at 1st and 2nd week postpartum (PP) in 50 Mehsana buffaloes. The difference and their association were seen in relation to postpartum diseases and reproductive performance.

Result: The glucose concentration significantly (P<0.05) decreased in 2nd week compared to 1st week postpartum in Mehsana buffaloes with anoestrous (>75 days PP), longer service period (>125 days) and postpartum disorders (Within 125 days). The BHBA and NEFA did not differ significantly between the time points or between the buffaloes suffering from postpartum disorders or not. The early postpartum glucose level can be used as a predictive indicator for buffaloes suffering from anoestrus, longer service period and postpartum disorders.

Key words: Buffalo, Early postpartum, Glucose, Reproductive performance.

INTRODUCTION

During the early postpartum period, buffaloes are often accompanied by retained fetal membranes (RFM), metritis, clinical endometritis (CE), milk fever, ketosis, mastitis, *etc.* (Dohoo *et al.*, 1983; Duffield *et al.*, 2009; LeBlanc, 2010). These disorders delay the resumption of ovarian cyclicity resulting in increased calving intervals in cattle and buffaloes. Approximately 75% of diseases in dairy cattle occur in the first month postpartum and 50% of dairy cattle suffer from metabolic and infectious diseases during the transition period (LeBlanc, 2010). Further, it has also been observed that buffaloes without postpartum disorders had problems of increased first postpartum estrus and service period (Sutaria, 2017). Increased insulin resistance due to negative energy balance (NEB) in the early postpartum period decreases blood glucose and insulin-like growth factors (IGFs), which affect folliculogenesis and ovulation (Lucy *et al.*, 2013). It has been reported that blood glucose within 1st week postpartum was the best indicator of diagnosis of metritis and endometritis (Bicalho *et al.*, 2017). Past research has extensively studied the association of NEFA and BHBA with postpartum diseases such as metritis, ketosis and displaced abomasum (Sepulveda-Varas *et al.*, 2015). Blood calcium level usually decreases during the first few weeks of calving. Decrease calcium is associated with RFM, milk fever and hypothalamo-pituitary-gonadal (HPG) axis function (Overton *et al.*, 2017). The cholesterol acts as a precursor of steroid hormones and indicates the circulatory adequacy of hormones responsible for normal oestrus (Ramakrishna, 1997). An increase in total protein levels is also associated with protein requirements for milk formation and immunoglobulin supply (Bremmer *et al.*, 2000; Mohri *et al.*, 2007). Most current research has focused on evaluating negative energy indices, NEFA and BHBA as predictors of postpartum diseases, while few studies have assessed the importance of glucose. Therefore, the study was designed to investigate associations between the metabolic indicators NEFA, BHBA, glucose, calcium, cholesterol and total protein during the early postpartum period, uterine diseases and the subsequent effect on reproductive performance.

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MATERIALS AND METHODS

The experiment was conducted from January 2018 to December 2019 at Livestock Research Station, Sardarkrushinagar. The freshly calved Mehasana buffaloes

(n=50) were included in the study and all the buffaloes were maintained under isomanagerial conditions. The buffaloes were in 1 to 5 parities with more than 8 L/day milk yield. Blood samples were collected by jugular venipuncture aseptically using 18-G needle in sterilized clot activators vacutainers during 1st and 2nd week postpartum. Serum was separated by centrifugation at 800 × g for 10 min and stored at -20 °C until analysis.

The blood glucose was estimated using a glucometer (On Call®, ACON Laboratories, Inc.), whereas calcium, total protein (TP) and cholesterol were analyzed from serum using commercially available diagnostic kits (AGAPPE Diagnostics Ltd., Agappe Hills, Dist. Ernakulam, Kerala, India) on semi-automatic biochemistry analyzer RX-50V (Micro Lab, India). The β-hydroxybutyrate (BHBA) and non-esterified fatty acid (NEFA) were estimated from serum by Enzyme Linked Immuno Sorbent Assay (Bioassay Technology Laboratory, Shanghai, China).

Estrus detection was done twice a day using a trained teaser bull. The postpartum buffaloes exhibiting estrus were inseminated using frozen semen. All the non-return animals were subjected to pregnancy diagnosis by trans-rectal palpation at 60-65 days of insemination. Postpartum reproductive variables such as first postpartum estrus duration (PPED), service period (SP) and pregnancy rate (PR) were recorded. The overall pregnancy rate was calculated by numbers of buffaloes became pregnant divided by the total number of insemination required. The types of calving (eutocia and dystocia) and postpartum reproductive disorders (RFM, milk fever, puerperal metritis, CE, mastitis and other ailments *viz.*, lameness) were also recorded. The diagnosis of metritis and clinical endometritis was based on characteristics described by Sheldon *et al.* (2006). Clinical mastitis was diagnosed by visual signs of inflammation such as clumpy, watery, bloody, or yellowish milk. Buffaloes were monitored for 600 days postpartum to record the postpartum estrus and service period. Buffaloes were retrospectively grouped based on the duration of postpartum estrus (PPED; ≤75 and > 75 days; El-Wishy, 2007), SP (≤125 and >125 days; Hafez and Hafez, 2000), types of calving (Eutocia and dystocia) and postpartum disorders (Yes and No).

Data were first checked for normal distribution by Shapiro-Wilk test. Descriptive analysis of estrus duration and service period was done. The two-way repeated measure ANOVA was used to see the effect of independent variables, postpartum disorders, PPED, SP and types of calving on blood glucose, BHBA, NEFA, calcium, cholesterol and TP considering fixed effects of independent variables and time and their interaction. The data analysis was carried out using a standard statistical software program (SPSS version 20, SPSS Inc, Chicago). The data were expressed as estimated marginal means with standard error. Differences were considered statistically significant at P≤ 0.05.

RESULTS AND DISCUSSION

All the buffaloes (n=50) were followed for 600 days postpartum. Out of 50 buffaloes, 45 exhibited the behavioral signs of estrus in 300 days. 03 buffaloes didn't show the estrus and 02 buffaloes were auctioned. Mean postpartum estrus duration (PPED) was 120.31(29-278) days. Out of 45 buffaloes, 36 conceived and confirmed pregnancy at two months through trans-rectal examination and 09 were non-pregnant or auctioned. The mean service period (SP) was 202.89±22.87 (41-528) days. Out of 50 buffaloes, 21 (42%) were suffered with postpartum disorders. The frequency distribution of different postpartum disorders in buffaloes is given in Table 1. The overall conception rate was 35% and service per conception was 2.83.

The blood glucose concentration was significantly (P=0.032) reduced at the 2nd week than the 1st week postpartum in buffaloes exhibited estrus at more than 75 days of calving. However, no significant difference in blood glucose concentration between the buffaloes exhibited estrus at ≤75 days and >75 days of calving at 1st and 2nd week postpartum (Fig 1). The TP, Ca, BHBA and NEFA did not differ significantly across the time or between the estrus exhibition at ≤75 or >75 days of calving (Fig1). The buffaloes having >125 days SP significantly (P=0.017) lowered blood glucose level at 2nd week as compared to 1st week postpartum (Fig 2). The circulatory level of calcium was significantly (P=0.007) reduced at the 2nd week in buffaloes having SP >125 days than SP ≤125 days (Fig 2). Whereas, no significant changes in the concentration of other blood metabolites between the buffaloes having SP ≤125 or >125 days at 1st and 2nd week PP.

The negative energy balance due to lactational stress during the early postpartum period affects the health of the bovines by altering the metabolic pathways (LeBlanc, 2010). The blood glucose is considered one of the energy status indicators in domestic animals (Hagawane *et al.*, 2009). The significantly higher blood glucose level at 1st week postpartum buffaloes suffering from postpartum reproductive disorders as well as longer PPED and SP in the current study. Bicalho *et al.* (2017) reported that the level of blood glucose was associated with the occurrence of postpartum reproductive diseases (metritis and clinical endometritis) increased SP (>150 d) in dairy cows. Similar to our results, BHBA and NEFA did not differ significantly between uterine diseased and healthy cows (Bicalho *et al.*, 2017).

Table 1: Frequency distributions of different postpartum disorders in buffaloes (n=50).

Postpartum disorders	N	%
Retained fetal membranes (RFM)	02	4
Puerperal metritis	02	4
Clinical endometritis	09	18
Mastitis	06	12
Other (lameness)	02	4

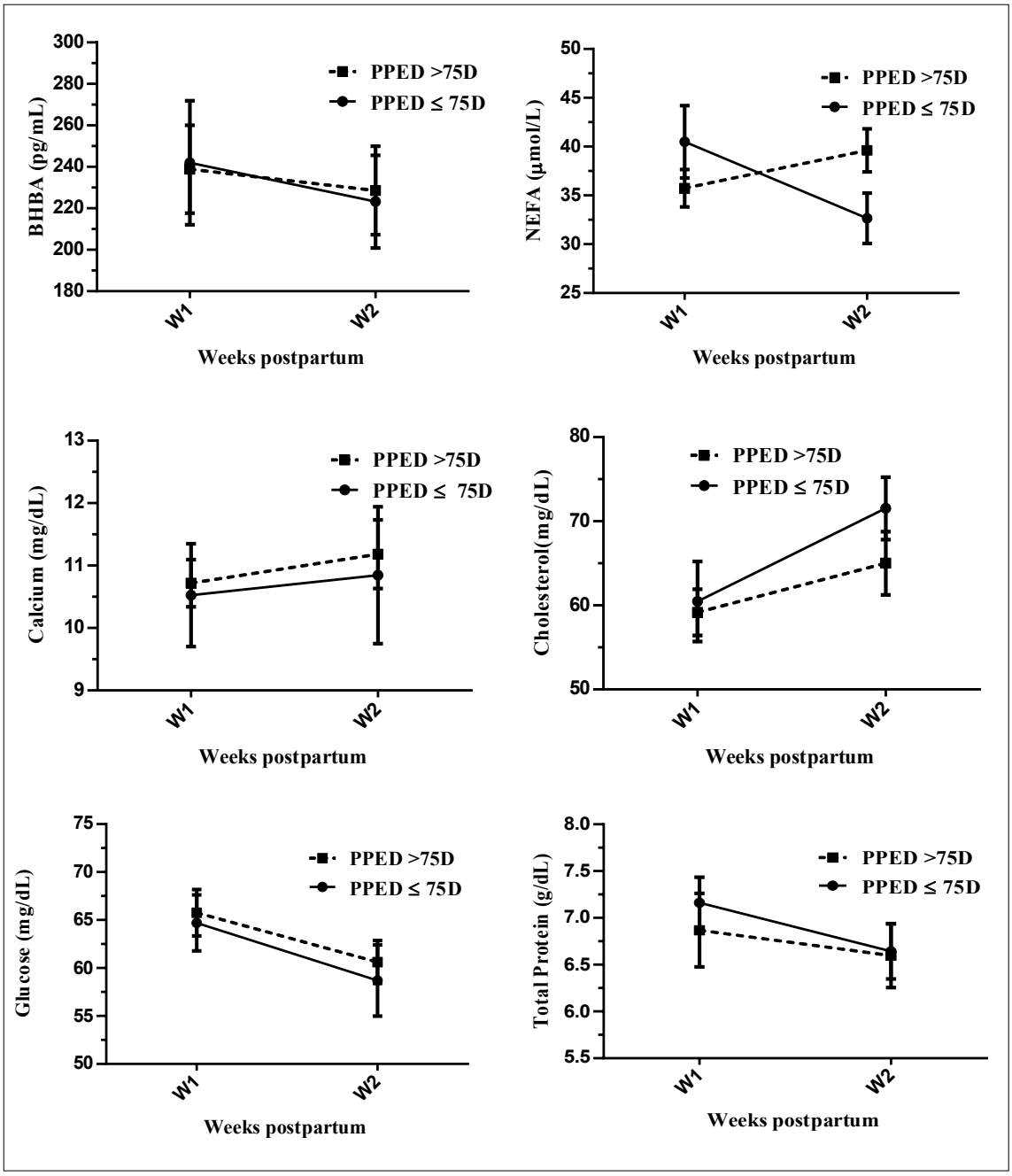

Fig 1: Blood biochemical profile during early postpartum period in buffaloes having postpartum estrus duration (PPED) ≤75 (n=13) and >75 days (n=37).

The blood glucose level was significantly reduced at 2nd week than the 1st week in buffaloes calved normally. The blood glucose was also significantly reduced in normally calved buffaloes than the dystocic buffaloes at 1st as well as 2nd week postpartum (Fig 3). However, the cholesterol concentration was significantly increased at the 2nd week than the 1st week postpartum in normally calved buffaloes, whereas, the corresponding figure did not differ significantly in dystocic buffaloes. The blood glucose concentration was also reduced significantly (P=0.006) at the 2nd week compared to the 1st week postpartum in buffaloes suffering from PP disorders. However, there were no significant changes in the blood glucose level at any time postpartum between buffaloes suffering from PP disorders or not (Fig 4).The significantly lower blood glucose level at 2nd week postpartum in subclinical endometritic buffaloes was observed by Jan *et al.* (2021). Contrary to the current study's findings, Singh *et al.* (2004) did not find a significant difference in blood

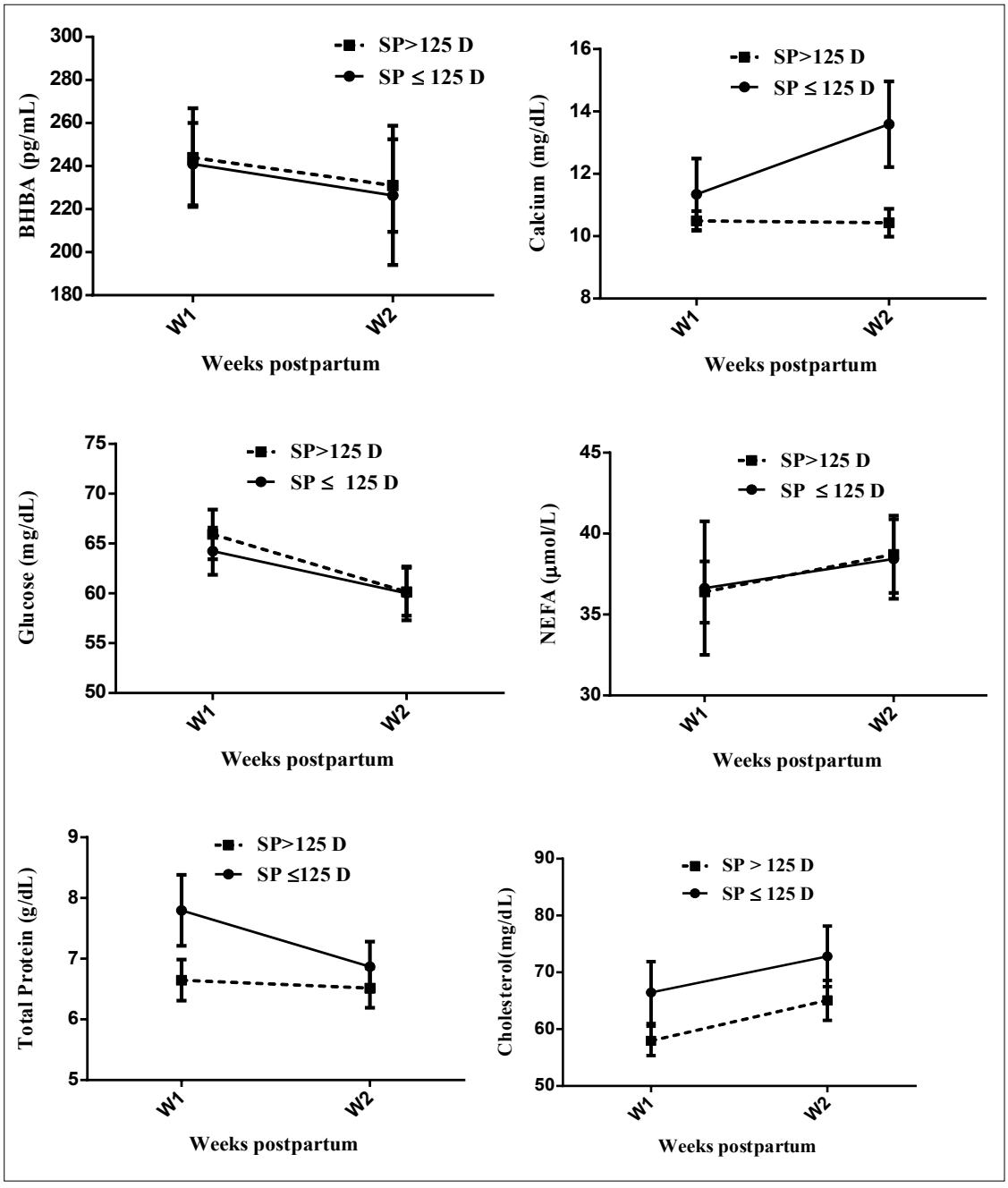

Fig 2: Blood biochemical profile during early postpartum period in buffaloes having service period ≤125 (n=13) and >125 days (n=37).

glucose level in buffaloes exhibited late postpartum estrus. Postpartum blood glucose was significantly higher in buffalo with dystocia than the normal calving in the current study, which agrees with Sutaria (2017).

The cholesterol concentration was significantly (P=0.004) increased at the 2nd week as compared to 1st week PP in buffaloes having no PP disorders. The calcium level was significantly lowered at 1st week than the 2nd week PP in buffaloes suffering from PP disorders. However, the BHBA and NEFA did not differ significantly between the time points or between the buffaloes suffering from PP disorders or not (Fig 4). This study's significantly lower TP in dystocic buffaloes is in synch with Sutaria (2017). Similarly, Amer and Hasem (2007) and Dhindsa *et al.* (2008) also reported the lower total protein in buffaloes suffering from dystocia. Similarly, Veena *et al.* (2015) found a non-significant difference in protein and cholesterol concentration in crossbred cows showed estrus within two months

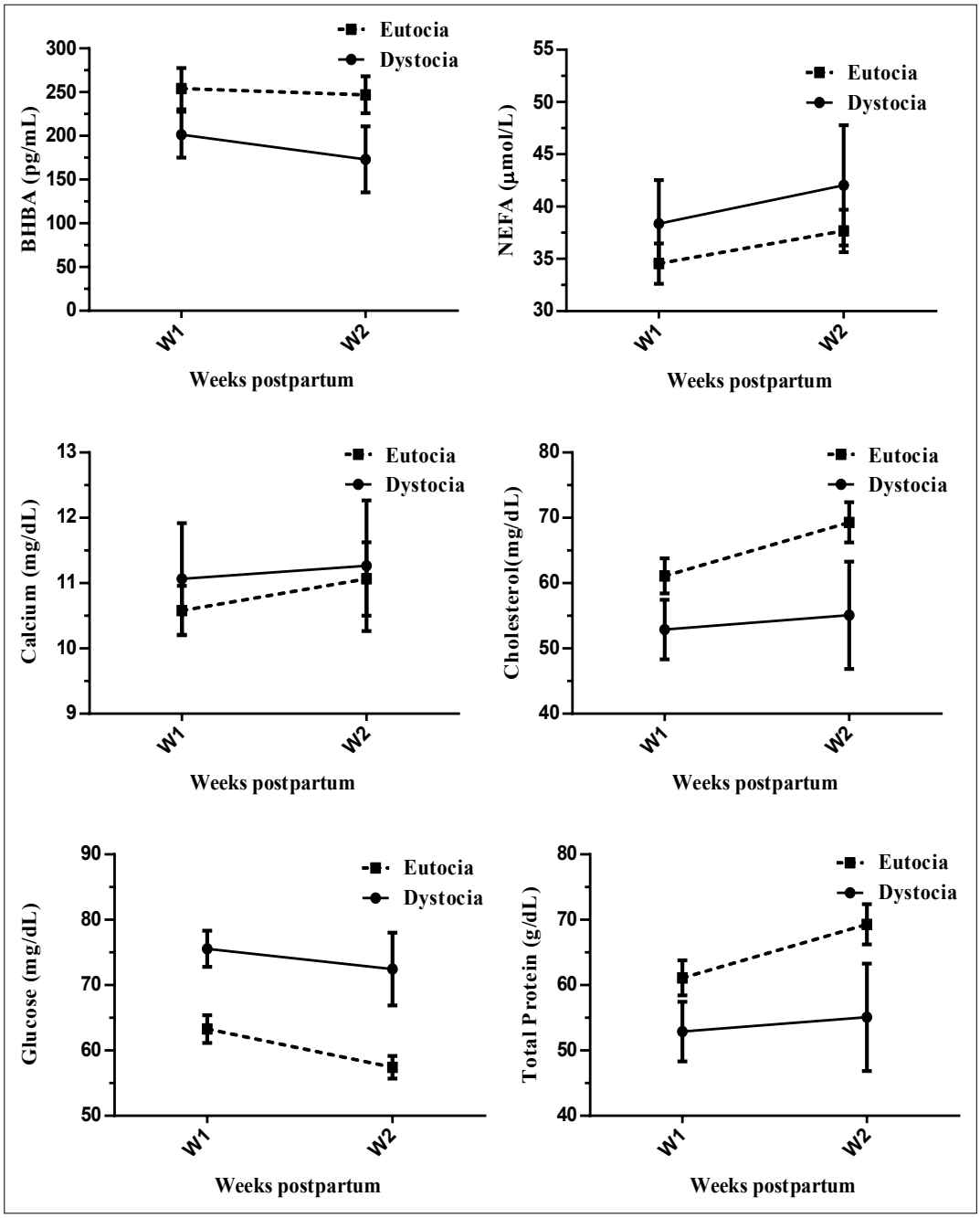

Fig 3: Blood biochemical profile during early postpartum period in dystocic (n=9) and eutocic (n=41) buffaloes.

postpartum and afterward. Reduced total protein may be due to reduced protein intake (Mahfooz *et al.*, 1994), tissue damage (Dhindsa *et al.*, 2005) and stress during dystocia (Bugalia *et al.*, 1996; Ghuman *et al.*, 1996). TP and cholesterol did not differ significantly in buffaloes exhibited the PPED within 75 days and SP less than 125 days in our study.

The calcium is involved in pulsatile GnRH secretion (Goor *et al.*, 2000) and steroidogenesis (Veldhuis and Klase, 1982). In our study, significantly lower calcium levels were found in buffaloes having longer SP at 2nd week postpartum; whereas, the significantly lower calcium at 1st week postpartum buffaloes suffering from postpartum disorders. The serum calcium concentration non-significantly reduced up to the 4th week postpartum in both normal and subclinical endometritic buffaloes (Jan *et al.*, 2021). Hedaoo *et al.* (2008) recorded lower calcium levels in acyclic buffaloes. A significantly lower calcium concentration was found in non-pregnant than the pregnant Murrah buffaloes (Yotov *et al.*, 2013). Sepulveda-Varas *et al.* (2015) also noted significantly lower calcium concentration in metritis cows at the first week postpartum.

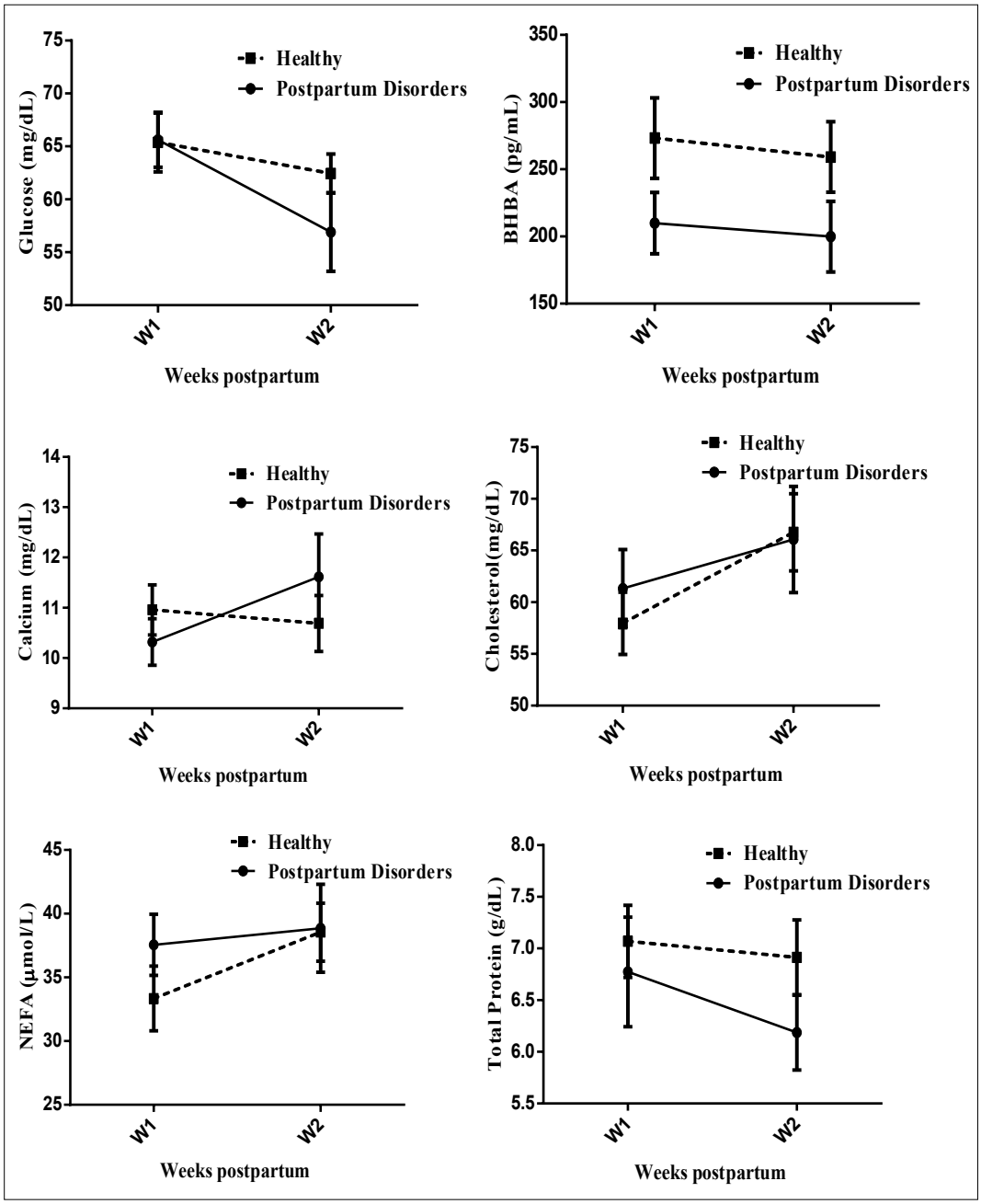

Fig 4: Blood biochemical profile during early postpartum period in healthy and buffaloes suffered with postpartum disorders.

CONCLUSION

The early postpartum glucose level can be used as a predictive indicator for buffaloes suffering from anoestrus, longer service period and postpartum disorders.

Conflict of interest: None.

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