SVU- International Journal of Veterinary Sciences, 6(1): 1-17, 2023
Print ISSN: 2535-1826 **Online ISSN: 2535-1877**

Research Article Open Access

Effects of Ketosis and Hypocalcemia on The Biochemical Parameters and Subsequent Postpartum Reproductive Performance in Buffaloes

Mohammed H. Ramadan[1, #], Nasra Ahmed M. Yousef[2, #], Alaa-Eldin Zain El-abdeen Mahmoud[3, *], Alaa E. B. Zeidan[1], Elsayed Mohammed Elsayed Mickdam[4, 5], Ahmed Ezzat Ahmed[2, 6], Ahmed Saad A. Hassaneen[2, *]

[1]Animal Production Research Institute (APRI), Dokki, Giza, Egypt, [2]Department of Theriogenology, Obstetrics, and Artificial Insemination, Faculty of Veterinary Medicine, South Valley University, 83523, Qena, Egypt, [3]Department of Theriogenology, Faculty of Veterinary Medicine, Sohag University, 82524, Sohag, Egypt, [4]Department of Nutrition, and Clinical Nutrition, Faculty of Veterinary Medicine, South Valley University, 83523, Qena, Egypt, [5]Educational and Research Animal Farm, Faculty of Veterinary Medicine, South Valley University, 83523, Qena, Egypt, [6]Biology Department, Faculty of Science, King Khalid University, 61413 Abha, Saudi Arabia.

Abstract

Water buffaloes (*Bubalus bubalis*) are mainly distributed in tropical and subtropical countries that include the Indian sub-continent and some Mediterranean countries such as Egypt. The transition period is the most stressful period for buffaloes, and it is considered as a turning point in the productive cycle from one lactation to the next and includes different metabolic, physiological, and nutritional changes. Metabolic disorders are common causes of lower productivity in buffaloes. Of these metabolic disorders, ketosis and hypocalcemia are most prevalent. This study aimed to study the ketosis- and hypocalcemia-related biochemical changes during the transition period and their impacts on the postpartum reproductive fertility in Buffaloes. Out of 120 total number of examined buffaloes, 40 buffaloes were used in this study; control group (n=10), hypocalcemia-affected (n=15), and ketosis-affected group (n=15). All buffaloes were subjected to thorough clinical and gynecological examination. Both urine and blood samples were collected from all groups. The amounts of ketone bodies were detected in urine. Biochemical parameters were evaluated including concentrations of glucose, triglycerides, cholesterol, non-esterified fatty acids (NEFA), calcium, phosphorus, sodium, potassium, albumin, total protein, urea and progesterone hormone (P4) in blood the following time-points (2-weeks prepartum, 1-week prepartum, partum, 1-week postpartum, and 2-weeks postpartum). Both hypocalcemia- and ketosis affected buffaloes had lower glucose, phosphorus, sodium and albumin and higher NEFA than control group. Hypocalcemia-affected buffaloes showed lower calcium and higher total protein than control group, while, ketosis-affected buffaloes showed lower cholesterol and total protein than control group. Moreover, metabolic disorders negatively affected the reproductive performance. Both ketosis and hypocalcemia significantly prolonged the duration to first estrus, increased both the number of days-open and the number of services per conception.

Keywords: Biochemical parameters, Hypocalcemia, Negative energy balance, Non-esterified fatty acids, Postpartum fertility, Reproductive Performance.

DOI: 10.21608/svu.2023.157726.1224 Received: August 22, 2022 Accepted: September 11, 2022 Published: January 3, 2023

*Corresponding Author: Alaa Eldin Zain Elabdin Mahmoud E-mail: zainmmn1960@gmail.com
and Ahmed Saad A. Hassaneen E-mail: ahmed.hassaneen@vet.svu.edu.eg

[#] These authors are equally contributed to this work

Citation: Ramadan et al., Effects of Ketosis and Hypocalcemia on The Biochemical Parameters and Subsequent Postpartum Reproductive Performance in Buffaloes. SVU-IJVS 2023, 6(1): 1-17.

Competing interest: The authors have declared that no competing interest exists.

Introduction

Water buffaloes (*Bubalus bubalis*) are mainly distributed in tropical and subtropical countries that include the Indian sub-continent and the Mediterranean countries. Buffaloes are raised for a variety of reasons, the most common of which are milk and meat production (Terzano et al., 2005). Rearing of water buffaloes is more suitable to harsh environment as they are more likely compete with and even surpass cows in their ability to adapt to sever climates (Webster and Wilson, 1980). Therefore, water buffaloes are of special economic and agricultural importance for the milk and meat production in Egypt (GOVS, 2005).

The transition period is the most stressful period for dairy animals (buffaloes and cows), since it caused numerous physiological changes for the beginning of lactation and as it is linked to oxidative stress, as well (Konvicna et al., 2015; Ambily et al., 2019). In addition, the dairy animals are susceptible to a variety of metabolic and hormonal changes during this period in the productive life (Tharwat et al., 2012). Although this period is relatively short (extends from 3 weeks before to 3 weeks after parturition), it considered the most critical period for dairy animals (Kessel et al., 2008). In fact, the transition period constitutes a turning point in the productive cycle from one lactation to the next and includes metabolic, physiological, and nutritional changes. Initiation of milk production caused more stress to the dairy animals than late gestation as the nutrients requirement by the mammary gland was many times greater than that of growing fetus (Tharwat et al., 2012). Consequently, dairy animals were highly susceptible to negative energy balance, fat mobilization, elevation of circulating non-esterified fatty acids (NEFA) and ketone bodies (Ingvarsten and Andersen, 2000; Seifi et al., 2007; Mohamed et al., 2015). Such metabolic imbalance makes the dairy animals more susceptible to production diseases such as ketosis, hypocalcemia, metritis, or mastitis during the transition period (Fleischer et al., 2001; van Knegsel et al., 2014; Hassaneen et al., 2020). Despite the fact that buffalos do not appear to go through the same stressful transition period as cows, significant physiological changes occur due to shifting from the pregnant, non-lactating state to the non-pregnant, lactating state (Mashek et al., 2001).

Metabolic disorders are common causes of lower productivity in buffaloes. Of metabolic disorders, ketosis considered one of most prevalent buffaloes' health and welfare issue that decreased the productivity in Egypt (Ghanem and El-Deeb, 2010). Such metabolic disorder is more likely occur in early lactation dairy cows and buffaloes when body reserves are used to support high energy demands (Youssef et al., 2010; Hassaneen et al., 2020). However, buffaloes have a less severe negative energy balance than dairy cattle due to lower milk production (Campanile et al., 2003). A study on Egyptian buffaloes reported that clinical ketosis has been recognized to be the most common wasting metabolic disorder (Youssef et al., 2010). In addition, ketosis due to extensive fat mobilization, was associated with low fertility (Walsh et al., 2007). Thus, detection of subclinical ketosis in the first 2 weeks of lactation was useful for management of herd problems and reproductive performance (LeBlanc, 2010). The development of ketosis does not appear to be related to negative energy condition alone; nevertheless, insufficient metabolic adaption appeared to play a role in the onset of ketosis (Herdt, 2000). Any condition that caused a reduction in feed intake during the peripartum period raised the risk of ketosis in dairy animals. For example, lameness and hypocalcemia have been associated with a higher risk of developing the disease (Curtis et al., 1983; Calderon and Cook, 2011). Milk fever (hypocalcemia) is a

metabolic disease that affects high-producing dairy buffaloes a few days prior to or after calving in the same way as it affects dairy cattle (Purohit et al., 2013). It accounts for substantial economic losses due to its association with decreased milk production, the cost of treatment and sometimes death (Hagawane et al., 2009). A significant association between hypocalcemia and both dystocia and retained fetal membranes in dairy animals had been reported. In this concern, hypocalcemia-affected animals were six times more likely to have dystocia and three times to have retained fetal membranes (Ferguson and Galligan, 1993). Such impairments that affects the functions of certain organs likely due to the of insufficiency of Ca required for the normal function of smooth muscle in organs such as the uterus, rumen and abomasum. One of the critical complications of hypocalcemia was uterine prolapse of the flaccid non-contractile uterus (Risco, 1994). Hypocalcemia-affected buffaloes were likely to develop similar changes to those reported in dairy cows, however, up to our knowledge, little descriptions are available, likely due to lower incidence in comparison to that in dairy cows. The strategy how to manage this imbalance was of great importance as they are closely related to clinical and subclinical postpartum metabolic diseases, and reproductive performance that could significantly affect profitability (Arm and Hammer, 2010).

Evaluation of hypocalcemia- and ketosis-related metabolic and biochemical parameters in the blood and/or urine of buffaloes during the transition period provide better understanding of these common metabolic disorders. Therefore, this study aimed to assess the hematological, biochemical and metabolic changes related to hypocalcemia and ketosis in buffaloes during the transition period and how much these disorders affect the subsequent fertility and productivity.

Material and methods

Ethical approval

This study was performed according to the Animal Ethics and Use Committee of the South Valley University for Veterinary Research, Qena, Egypt and all procedures were approved by the Department of Theriogenology, Obstetrics, and Artificial Insemination, Faculty of Veterinary Medicine, South Valley University (FC/No. 232/05.02.2018) and by the Ethical Research Committee, Faculty of Veterinary Medicine, South Valley University (Approval No. 59/18.09.2022). The present study was conducted at Animal Production Research Farm, Animal Production Research Institute, Beni Suef, Egypt, located at 32 m above mean sea level, latitude 29.03° N and longitude 31.10° E.

Out of 120 total number of examined buffaloes during transition period, 40 female buffaloes were used in this study. All animals were subjected to general and gynecological examination. Body condition score (BCS) was determined according to scale of 1 to 5, with increments of 0.25. Buffaloes were housed in free stalls, fed a diet formulated according to standard guidelines, with an ad libitum access to water.

Buffaloes were divided into three groups; control group included 10 buffaloes (parity; 3.55 ± 0.31, BCS of 3.10± 0.15), hypocalcemia-affected group included 15 buffaloes (parity; 3.13 ± 0.31, BCS of 3.15± 0.11), and ketosis-affected group included 15 buffaloes (parity; 3.00 ± 0.35, BCS of 3.07± 0.10). The control group had normal calving and absence of any post-parturient metabolic disorders. The hypocalcemia-affected group showed symptoms of restlessness, excitability, protruded tongue, decreased ruminal motility, and lies down with head towards the flank, while the ketosis-affected group included those with prepartum symptoms of decreased appetite, decreased milk

production, profuse salivation, abnormal gait, grasping hard objects, and lack of alertness.

All Buffaloes were subjected to thorough clinical and gynaecological examination and Buffaloes with any pathological changes in the reproductive tract, abnormal vaginal discharge, or signs of systemic illness were excluded. For each animal, a data sheet was recorded including animal number, age, parity, BCS, and general health condition. Pregnancy diagnosis was performed at 45-60 days post insemination and the fertility indices were assessed using interval from calving to the first estrus (days), number of services per conception (NSC) and days open as the intervals from calving to conception (days).

Analysis of metabolic status indices

For determination of ketone bodies in urine, urine samples were aseptically collected from all buffaloes every week starting from 4 weeks before the expected day of calving till 2 weeks postpartum. The samples' collection was performed using a disposable collecting plastic sheath gently inserted inside the external urethral orifice visualized by a vaginoscope (Ramadan et al., 2020). Ketone bodies level was estimated by using Medi-Test Comb-10 kits. The amount of ketone bodies was categorized as; none (–), traces (+/–), positive (+) and strongly positive (++/+++).

Blood samples were collected at the same time of urine sampling. The samples were collected from jugular vein using 9-mL evacuated tubes to determine calcium (Ca), phosphorus (P), sodium (Na), potassium (K), cholesterol, triglycerides NEFA, albumin, total protein, and urea. Glucose samples were collected in fluoride plasma tubes to avoid glycolysis during storage. After sampling, serum samples were allowed to clot for around 2 hours at room temperature, whereas plasma samples were kept at 7°C before being centrifuged at 2,000 × *g* for 10 min at room temperature. Thereafter, samples were kept at−20°C until further analyses. For analysis of biochemical parameters, the following time-points were used; 2-weeks prepartum, 1-week prepartum, day of calving, 1-week postpartum, and 2-weeks postpartum. Biochemical analyses were performed by using automatic absorption spectrophotometer (Erba Chem 7, Erba Diagnostics Mannhein GmbH, Germany) and the specific kits (Spinreact, SA/SAU. Ctra. Santa Coloma, Sant Esteve De Bas, Girona, Spain) at different wavelength (340 nm for P levels (mg/dl), 505nm for glucose (mg/dl), and cholesterol (mg/dl) levels, 546 nm for triglyceride (mg/dl) and total protein levels (g/dl), 570 nm for Ca levels (mg/dl), and 578 nm for K (mmol/l), and urea levels (mg/dl), 630nm for Na (mmol/l), and albumin (g/dl) according to the manufacturer's instructions. Serum NEFA (mmol/l) were assessed using NEFA kits (Colorimetric, Randox Reagents, Randox Laboratories Ltd. London, London, United Kingdom). Serum P4 levels (ng/dl) were measured using radioimmunoassay kits while assessment haematological parameters were performed by measuring of haemoglobin concentrations (Hb; g/dl) and total leukocyte count (WBCs).

Statistical analyses:

All data were expressed as mean± SEM. Statistical analyses were conducted according to SPSS. Statistical significance was determined by analysis variance (ANOVA) multiple range and simple regression. Statistically significant differences values were set at $P \leq 0.05$.

Results

Fertility parameters:

The duration to first estrus (d; mean± SEM) was approximately 36 d longer ($P \leq 0.05$) in the hypocalcemia and ketosis-affected buffaloes (Table 1).Similarly, the

days-open were 23 d longer in the hypocalcemia- and 36 d longer in ketosis-affected buffaloes in comparison to control group ($P \leq 0.05$). In addition, the required number of services per conception (NSC; mean± SEM) was higher by approximately 29% ($P < 0.05$) in the hypocalcemia- and 64% in ketosis-affected buffaloes compared to control group.

Amounts of ketone bodies in urine of the hypocalcemia-, ketosis-affected and control buffaloes:

Amounts of ketone bodies in urine in all the hypocalcemia-, ketosis-affected and control buffaloes varied according to the sampling time-point. All control group buffaloes had (+/-) ketone bodies amounts in urine at the prepartum period; these amounts were increased to (+) at the day of calving and postpartum period (Fig. 1).

In hypocalcemia-affected buffaloes, the amount of ketone bodies in urine varies between animals, it was (+) in 13 buffalos, (++) in 2 buffalos at the prepartum timepoints, while at the day of calving, the amount of ketone bodies was (+) in 12 buffalos, (++) in 3 buffalos and at the postpartum timepoints, 8 buffaloes had (+), 4 buffalos had (++), and 3 buffalos had (+++) amounts of ketone bodies (Fig. 1).

In ketosis-affected buffaloes, the amount of ketone bodies in urine was (+) in 12 buffalos, (++) in 3 buffalos at the prepartum timepoints while at the day of calving, the amount of ketone bodies was (+) in 8 buffalos, (++) in 7 buffalos, at 1-week postpartum timepoint, 5 buffalos had (++), and 10 buffalos had (+++) amount of ketone bodies in urine, much more increase in the amount of ketone bodies in urine that 3 buffalos had (++), and 12 buffalos had (+++) amount of ketone bodies in urine (Fig. 1).

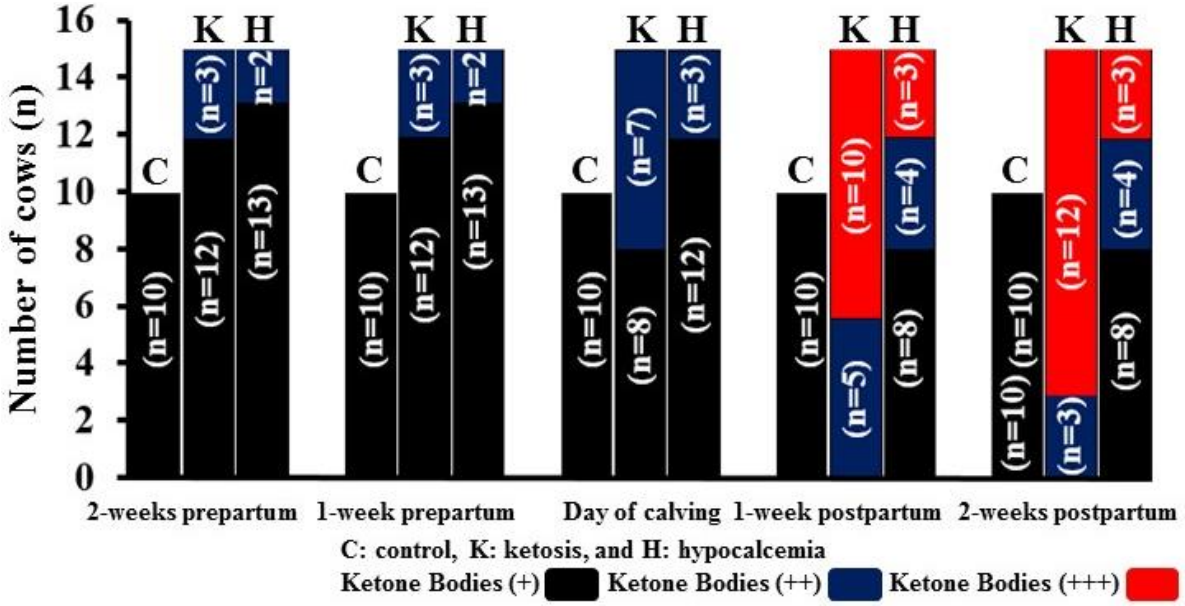

Fig. 1. Amount of ketone bodies in urine in the control (C), hypocalcemia-(H), and ketosis-affected (K) buffaloes at 1-week prepartum, 2-weeks prepartum, day of calving, 1-week postpartum, and 2-weeks postpartum.

Table 1. Fertility parameters including duration to 1st estrus (d), days-open (d), and number of services per conception (NSC; n) in control, hypocalcemia-, and ketosis-affected buffaloes.

Fertility parameter / Group	Control	Hypocalcemia	Ketosis
Duration to 1st estrus (d)	40.30± 2.08	75.20± 3.46*	76.86± 4.28*
Days-open (d)	66.50± 3.36	89.53± 4.04*	102.53± 54.21*
NSC[1] (n)	1.30± 0.15	1.67± 0.16*	2.13± 0.13*

Values are expressed as Mean± SEM. (*) means significant at ($P \leq 0.05$).

Haematological parameters in the hypocalcemia-, ketosis-affected and control buffaloes:

The concentrations of Hb were decreased ($P \leq 0.05$) in the blood of hypocalcaemia- and ketosis-affected buffaloes at day of calving and 1-week postpartum in comparison to 2-weeks prepartum. On the other hand, Hb concentrations were increased ($P \leq 0.05$) in the control group at day of calving and 2-week postpartum in comparison to 2-weeks prepartum (Fig. 2A).

The WBCs counts were lower ($P \leq 0.05$) in the blood of ketosis-affected buffaloes at day of calving and both postpartum timepoints in comparison to 2-weeks prepartum timepoint, while blood of control and hypocalcaemia-affected buffaloes showed higher WBCs counts($P \leq 0.05$) at day of calving in comparison to 2-weeks prepartum timepoint (Fig. 2B).

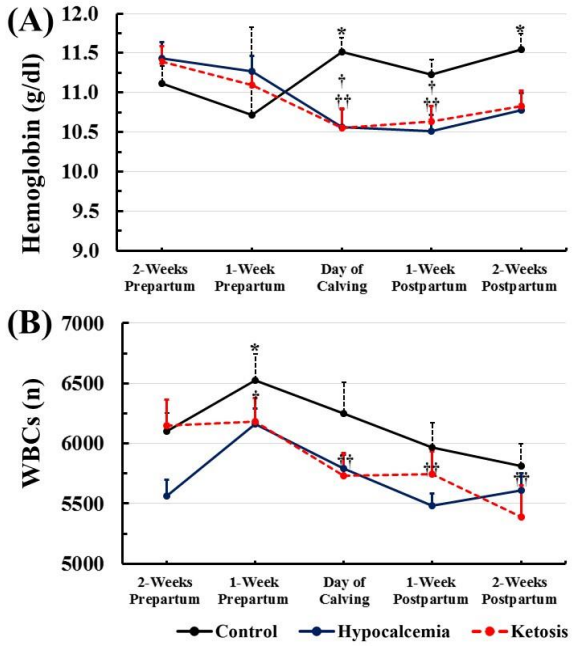

Fig. 2. Haematological parameters; (A) hemoglobin concentrations (g/dl), and (B) WBCs counts (n) in the control (black), hypocalcemia (blue), and ketosis-affected (red) buffaloes at 1-week prepartum, 2-weeks prepartum, day of calving, 1-week postpartum, and 2-weeks postpartum. Significant difference between the measured value at certain timepoint and that at 2-weeks prepartum timepoint are expressed as (*) in the Control, (†) in the hypocalcemia-, and (††) in the ketosis-affected buffaloes

Biochemical parameters in the hypocalcemia-, ketosis-affected and control buffaloes:

Energy status parameters:

Plasma glucose levels in the hypocalcemia-affected buffaloes were lower at the day of calving, and 1-week postpartum timepoints than those at 2-weeks prepartum ($P \leq 0.05$). Moreover, the plasma glucose levels in the hypocalcemia-affected buffaloes at 2-weeks prepartum, day of calving and 1-week postpartum time-points were lower ($P \leq 0.05$) than those timepoints in the control buffalos (Fig. 3A), while the plasma glucose levels in the ketosis-affected buffaloes were lower at the day of calving, and both postpartum timepoints than those at 2-weeks prepartum ($P \leq 0.05$). Moreover, the plasma glucose levels in the ketosis-affected buffaloes at 2-weeks prepartum, day of calving and both postpartum time-points were lower ($P \leq 0.05$) than those time-points in both the control and hypocalcemia-affected buffaloes (Fig. 3A). The lowest glucose levels recorded at 1-week postpartum was 16.8 mg/dl.

Serum cholesterol levels in the hypocalcemia- and ketosis-affected buffaloes followed the same pattern of plasma glucose levels (Fig. 3B), while no changes were detected between the serum cholesterol levels in the hypocalcemia-affected and the control buffaloes at all time-points (Fig. 3B). Moreover, the serum cholesterol levels in the ketosis-affected buffaloes at day of calving and both postpartum time-points were lower ($P \leq 0.05$) than those in the control and hypocalcemia-affected buffaloes (Fig. 3B).

Serum triglycerides levels in the both hypocalcemia-affected and ketosis-affected buffaloes were lower ($P \leq 0.05$) at the day of calving, and both postpartum timepoints than those at 2-weeks prepartum (Fig. 3C). Moreover, the serum triglycerides levels in the hypocalcemia-affected buffaloes were lower at the day of calving and were higher at 1-week prepartum timepoint than those in the control buffaloes ($P \leq 0.05$), while the serum triglycerides levels in the ketosis-affected buffaloes at day of calving and 1-week postpartum time-points were lower ($P \leq 0.05$) than those in the control and hypocalcemia-affected buffaloes (Fig. 3C).

Serum NEFA levels in the hypocalcemia-affected buffaloes were higher ($P \leq 0.05$) at the day of calving, and 1-week postpartum timepoints than those at 2-weeks prepartum timepoint (Fig. 3D). Moreover, serum NEFA levels in the ketosis-affected buffaloes were higher ($P \leq 0.05$) at the day of calving, and both postpartum timepoints than those at 2-weeks prepartum timepoint peaking at 2-weeks postpartum (Fig. 3D). The serum NEFA levels in the hypocalcemia-affected buffaloes at day of calving and both postpartum time-points were higher ($P \leq 0.05$) than those in the control buffaloes (Fig. 3D).

The serum NEFA levels in the ketosis affected buffaloes at day of calving were higher ($P \leq 0.05$) than those in the control buffaloes and at both postpartum time points were higher ($P \leq 0.05$) than those in the control and hypocalcemia-affected buffaloes (Fig. 3D).

.

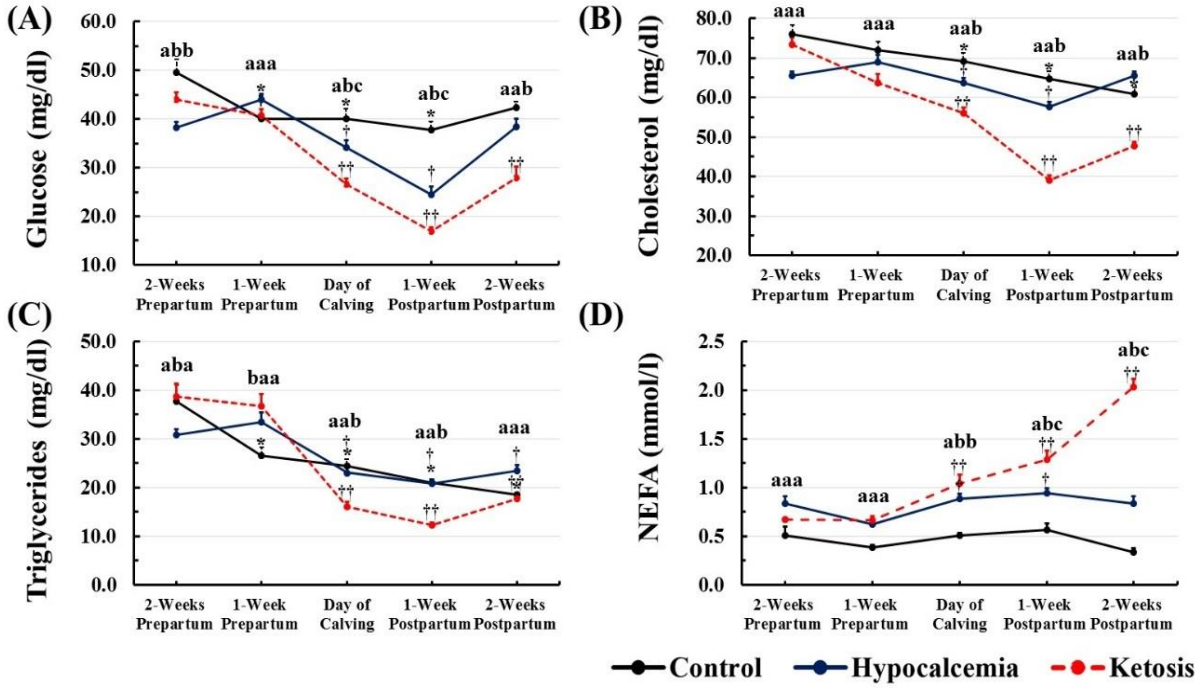

Fig. 3. Energy related parameters; (A) Glucose (mg/dl), (B) Cholesterol (mg/dl), (C) Triglycerides (mg/dl), and (D) Non-esterified fatty acids (NEFA; mmol/l) in the control (black), hypocalcemia (blue), ketosis-affected (red) buffaloes at 1-week prepartum, 2-weeks prepartum, day of calving, 1-week postpartum, and 2-weeks postpartum. Significant difference between the measured value at certain timepoint and that at 2-weeks prepartum timepoint are expressed as (*) in the control, (†) in the hypocalcemia-, and (††) in the ketosis-affected buffaloes. Different letters mean significant difference between groups at the same timepoint where the first letter is for the control, middle letter for the hypocalcemia and last letter for the ketosis.

Metabolism related minerals:

Serum Ca levels in the hypocalcemia-affected buffaloes were lower ($P \leq 0.05$) at day of calving with sharp decreased at 1-week postpartum and lasting low at 2-weeks postpartum time-point in comparison to those at 2-weeks prepartum timepoint. Moreover, the values of Ca levels in the hypocalcemia-affected buffaloes were lower at all time-points ($P \leq 0.05$) than those in the control buffaloes. No changes were detected between serum Ca levels at all time-points in the ketosis-affected group and no changes were detected between serum Ca levels in the ketosis-affected group and those in the control group at all time-points (Fig. 4A).

Serum P levels in the hypocalcemia-affected buffaloes were lower at postpartum time-points ($P \leq 0.05$) than those at 2-weeks prepartum. Moreover, the hypocalcemia-affected buffaloes showed lower serum P levels at 2-weeks prepartum and 1-week postpartum ($P \leq 0.05$) than those in the control buffaloes (Fig. 4B). Serum P levels in the ketosis-affected buffaloes were lower at the day of calving and both postpartum time-points ($P \leq 0.05$) than those at 2-weeks prepartum. The values of P levels in the ketosis-affected buffaloes were significantly lower at both postpartum timepoints than those in the control buffaloes (Fig. 4B).

Serum Na levels in the hypocalcemia-affected buffaloes were lower at 1-week prepartum, day of calving, and 1-week postpartum time-points ($P \leq 0.05$) than those at 2-weeks prepartum. Moreover, the values of Na levels in the hypocalcemia-affected buffaloes were lower at all timepoints ($P \leq 0.05$) than those in the control buffaloes. The values of Na levels in the ketosis-affected buffaloes were lower at 1-week prepartum, day of calving, and both postpartum timepoints ($P \leq 0.05$) than those in the control buffaloes (Fig. 4C).

Serum K levels in the both hypocalcemia-affected and ketosis-affected buffaloes were not changed throughout the whole period of sampling. Moreover, the values of K levels in the both hypocalcemia-affected and ketosis-affected buffaloes were lower at 1-week prepartum, day of calving, and both postpartum timepoints ($P \leq 0.05$) than those in the control buffaloes (Fig. 4D).

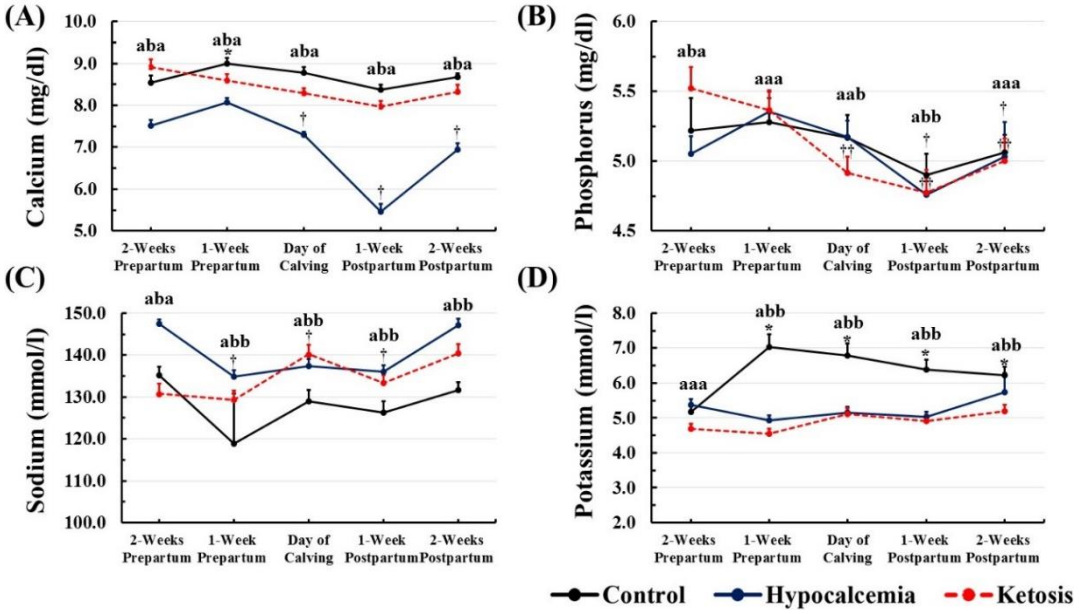

Fig. 4. Metabolism related minerals; (A) Calcium (Ca; mg/dl), (B) Phosphorus (P; mg/dl), (C) Sodium (Na; mmol/l), and (D) Potassium (K; mmol/l) in the control (black), hypocalcemia (blue), ketosis-affected (red) buffaloes at 1-week prepartum, 2-weeks prepartum, day of calving, 1-week postpartum, and 2-weeks postpartum. Significant difference between the measured value at certain timepoint and that at 2-weeks prepartum timepoint are expressed as (*) in the control, (†) in the hypocalcemia-, and (††) in the ketosis-affected buffaloes. Different letters mean significant difference between groups at the same timepoint where the first letter is for the control, middle letter for the hypocalcemia and last letter for the ketosis

Blood proteins and protein metabolites:

Serum albumin levels in the hypocalcemia-affected buffaloes were lower at 2-weeks prepartum and higher at postpartum timepoints ($P \leq 0.05$) than those in the control buffaloes (Fig. 5A), while the values of albumin levels in the ketosis-affected buffaloes were significantly lower at day of calving, and both postpartum timepoints ($P \leq 0.05$)than those in the control buffaloes (Fig. 5A).

Serum total protein levels in the hypocalcemia-affected buffaloes were higher at postpartum timepoints than those in the control buffaloes (Fig. 5B). The serum total protein levels in the ketosis-affected buffaloes were significantly lower at day of calving, and 1-week postpartum timepoints than 2-weeks-prepartum timepoint (Fig. 5B).

Serum urea levels in the hypocalcemia-affected buffaloes were significantly lower at prepartum and day of calving timepoints than those in the control buffaloes (Fig. 5C). The serum urea levels in the ketosis-affected buffaloes were significantly increased at 1-week postpartum timepoint than 2-weeks-prepartum timepoint and these value at the postpartum timepoints were significantly higher than control buffaloes (Fig. 5C).

Serum P4 levels (ng/dl) in the hypocalcemia-, ketosis-affected, and control buffaloes:

Serum P4 hormone levels in the both hypocalcemia- and ketosis-affected buffaloes was lower at day of calving ($P \leq 0.05$) than those in the control group (Fig. 6). However, serum P4 hormone levels in the both hypocalcemia- and ketosis-affected buffaloes were significantly higher than those in the control group (Fig. 6).

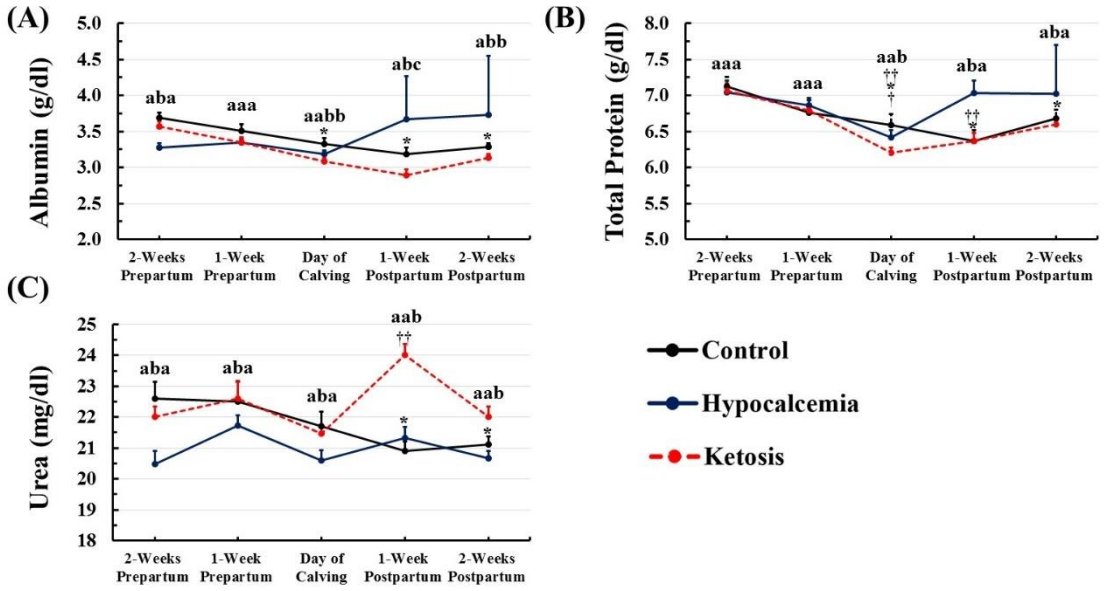

Fig. 5. Protein and protein metabolites; (A) Albumin (g/dl), (B) Total protein (g/dl), and (C) Urea (mg/dl) in the control (black), hypocalcemia (blue), ketosis-affected (red) buffaloes at 1-week prepartum, 2-weeks prepartum, day of calving, 1-week postpartum, and 2-weeks postpartum. Significant difference between the measured value at certain timepoint and that at 2-weeks prepartum timepoint are expressed as (*) in the control, (†) in the hypocalcemia-, and (††) in the ketosis-affected buffaloes. Different letters mean significant difference between groups at the same timepoint where the first letter is for the control, middle letter for the hypocalcemia and last letter for the ketosis.

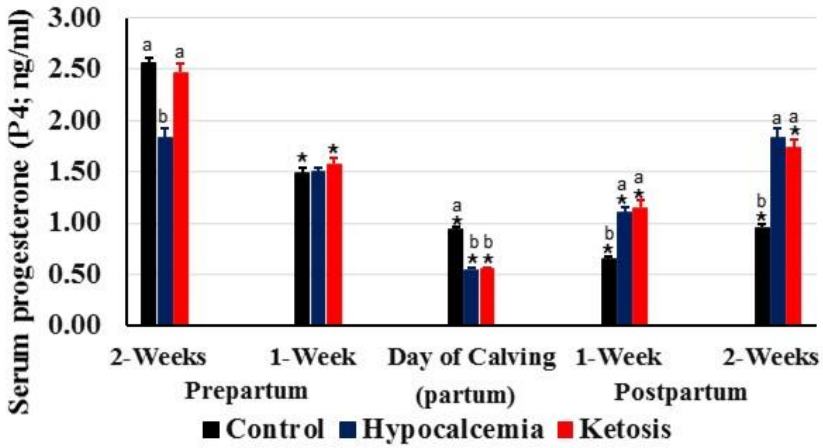

Fig. 6. Serum progesterone (P4) levels (ng/ml) in the control (black), hypocalcemia (blue), ketosis-affected (red) buffaloes at 1-week prepartum, 2-weeks prepartum, day of calving, 1-week postpartum, and 2-weeks postpartum. Significant difference between the measured value at certain timepoint and that at 2-weeks prepartum timepoint are expressed as (*****). Different letters mean significant difference between groups at the same timepoint where the first letter is for the control, middle letter for hypocalcemia and last letter for the ketosis.

Discussion

Due to the enormous demand for buffalos' milk- and meat-based products, production of these animals now closely resembles that of cattle in many countries. More research should be done with a clear focus on the transition between various physiological stages if this species is to perform optimally under the stress of intensive production systems (Bauman, 2000; Campanile et al., 2006). Buffaloes exhibited a very different metabolic pattern from other ruminants (Fiore et al., 2017), and there were only been few investigations on the physiology of the transition period in buffaloes (Abdulkareem, 2013; Fiore et al., 2017; Abdelrazek et al., 2018). Quick alterations key metabolites such as NEFA, glucose and Ca have been linked to production and may be used to diagnose metabolic and reproductive disorders in dairy cow. However, such information on buffaloes is still scarce. Therefore, the findings of the current study determined the changes in the hypocalcemia- and ketosis-related biochemical parameters in buffaloes during the transition period and how these metabolic disorders negatively effect on their reproductive performance.

Urinary specimens offered a definite advantage over other biofluids (Gowda et al., 2008). Thus, in the current study, urine was used as a diagnostic tool for ketosis affected buffaloes. As expected, all ketosis buffaloes had high amount of urine ketone bodies than that in the control. In addition, buffaloes with hypocalcemia had high amount of ketone bodies in urine (but lower than that in ketosis), this was also reported by Magnus and Lali, (2009). The ketosis and hypocalcemia-affected buffaloes had lower Hb concentrations at the day of parturition and postpartum period. Lower Hb concentrations could be attributed to the physiological losses of blood during parturition and probably as a result of low feed intake or inappetence. Ketone bodies among the metabolites linked to negative energy balance appeared to have number of detrimental effects on immunological processes (Overton and Waldron, 2004). In addition, the concentration of NEFA in blood also might affect immune function (Hammon et al., 2006). This could explain

the lower WBCs level in ketosis affected buffaloes in the current study. In hypocalcemia-affected buffaloes, low WBCs count could be attributed to impaired uterine immunity caused by decrease of muscles contraction due to Ca deficiency.

Biochemical analysis revealed that ketosis-affected buffaloes had lowered levels of plasma glucose, serum cholesterol, triglycerides, total protein and albumin. These findings were in agreement with those reported by Sharma and Rakesh, (2001); Elitok et al., (2006); Yameogo et al., (2007). On the contrary, in agreement with Nazifi et al., (2008); Farag and Metwally, (2012) levels of NEFA and ketones were elevated markedly in ketosis affected buffaloes. This drop in glucose levels could be the result of energy deprivation, particularly in the early stages of lactation when the mammary gland has to use glucose at a high rate (Bremmer et al., 2000). Low glucose levels trigger the mobilization of fat to support a negative energy balance, which raised NEFA and ketone levels, which were crucial energy sources when carbohydrate levels are low (Dann et al., 2005; Padilla et al., 2005). The reduction of triglycerides in ketonic buffaloes could be explained by an increase in hepatic cell lipid uptake that results in the development of hepatic lipidosis and a concurrent decrease in hepatic triglyceride output, which lowers the level of circulating triglycerides (Vermunt, 2003). Moreover, the capacity of liver lipoprotein synthesis was low in ketosis affected buffaloes which could explain low plasma protein and albumin in the current study (Farag and Metwally, 2012).There were notable changes in different blood parameters related with the energy status in hypocalcemia-affected buffaloes in the current study. The most important result was the lower plasma glucose levels is at calving and postpartum periods than the prepartum period which attributed to negative relationship between hypocalcemia and normal smooth muscle contractility in the ruminant stomach which led to decreased in appetite (Huber et al.,1981). The energy insufficiency due to low glucose levels reported in this study likely resulted in some metabolic events that increased fat mobilization (Meena et al., 2022). Consequently, the level of NEFA was elevated in the hypocalcemia-affected buffaloes. In this context, Bremmer et al., (1999) reported that one third of cows developed milk fever when NEFAs<1.2 mEq/L. Therefore, the present study revealed a strong relation between the occurrence of hypocalcemia and the energy status of the buffaloes during the transition period.

When compared to the control group, the blood calcium level in ketosis-affected buffaloes did not differ markedly. This result was in line with those of Akhtar et al., (2007), Akhtar et al., (2008), Khan and Akhtar, (2007) and Farag and Metwally, (2012). On the other hand, hypocalcemia-affected buffaloes showed sever decrease in serum Ca and P levels. These findings concurred with those that were previously recorded (Abd El-Raof and Mobarak, 2006; Farag and Metwally, 2012).

Hypocalcemia, ketosis, and other dietary changes affect reproductive capacity (Miqueo et al., 2019). Low progesterone levels have been linked to negative energy balance and have been demonstrated to have a deleterious impact on the outcome of early pregnancy (Sammad et al., 2022). According to the literature, postpartum nutritional metabolic disorders are the main causes behind post-parturient reproductive abnormalities in dairy animals (Cardoso et al., 2020). Recent research has revealed connections between reproductive performance and energy metabolism throughout the periparturient phase (Butler et al., 2006; Roche et al., 2009; Ospina et al., 2010). In a large field investigation, elevated blood levels of NEFA during the transition period were

linked to poor reproductive performance (Ospina et al., 2010).In addition, postpartum negative energy balance and high NEFA concentrations have demonstrated evidence for greater levels of inflammation resulting in alterations in uterine functions (Wathes et al., 2007; Zhang et al., 2018; Velázquez et al., 2019). It is clear that postpartum negative energy balance might be considered a primary cause of a variety of postpartum production disorders (Sammad et al., 2022). In the present study buffaloes affected with hypocalcemia and ketosis suffered from negative impact on fertility parameters appeared as prolonged the interval to first postpartum estrus and higher number of days open and higher required NSC. This attributed to the period of NEB with the consecutive delay of the beginning of reproductive functions with fertility reduction (Butler, 2000).

Conclusion

In conclusion, metabolic disorders significantly impaired both hematological and biochemical parameters in blood and urine of the affected buffaloes during the transition period. Both hypocalcemia- and ketosis affected buffaloes had lower levels of glucose, P, Na and albumin and higher levels NEFA in comparison to the control group. Hypocalcemia-affected buffaloes showed lower level of Ca and higher level of total protein in comparison to the control group, while, ketosis-affected buffaloes showed lower levels of cholesterol and total protein in comparison to the control group. Moreover, metabolic disorders negatively affected the reproductive performance that both ketosis and hypocalcemia significantly prolonged the duration to first estrus, significantly increased both the number of days-open and the NSC.

Conflict of interest statement

The authors declare that they have no conflict of interest.

References

Abd El-Raof YM, Mobarak MG (2006). Field studies, Haemato-biochemical and Histopathololgical examination on some cases suffering from milk fever in cows. 8th Sci. Vet. Med. Zag. Conference, Hurghada.

Abdelrazek H, Ismail TA, El-Azzazi FE, Elsayed DH (2018). Hematological and Metabolic Alterations in Egyptian Buffaloes during Transition Period. Egy. Acad. J. Biol. Sci., 10(1):69-78.

Abdulkareem T (2013). Some hematological and blood biochemical attributes of Iraqi riverine buffaloes (Bubalus bubalis) around calving and postpartum periods. Al-Anbar J. Vet. Sci, 6: 143-150.

Akamatsu HY, Saitoh K, Miyake Y, Ohba, Nakashima K (2007). Change of serum 3-methylhistidine concentration and energy-associated metabolites in dairy cows with ketosis. J. Vet. Med. Sci., 69(10): 1091-1093.

Akhtar MZ, Khan A, Javaid A (2008). Patho-biochemical changes in buffaloes (Bubalus bubalis) suffering from parturient haemoglobinuria. Pakistan Vet. J., 28(3): 139-143.

Akhtar MZ, Khan A, Sarwar M, Javaid A (2007). Influence of Soil and Forage Minerals on Buffalo (Bubalus bubalis) Parturient Haemoglobinuria. Asian-Aust. J. Anim. Sci., 20(3): 393-398.

Ambily TR, Beena V, Karthiayini K, Ramnath V, Uma R Siddaramesh A (2019). Effect of supra-nutritional supplementation of vitamin E and/or selenium on oxidant-antioxidant

profile in transition dairy cows. The Pharma Innovation J., 8(8): 97-106.

Arm, Hammer (2010). High Plains Dairy Conference-Elliot Block, PhD Animal Nutrition Church & Dwight Co.,).

Bauman DE (2000). Regulation of nutrient partitioning during lactation: Homeostasis and homeorhesis revisited. In: Cronje PB, editor. Ruminant physiology: digestion, metabolism, growth and reproduction. Wallingford, UK: CAB International; 2000. p. 311-28.

Bremmer DR, Christensen JO, Grummer RR, Rasmussen FE, Witbank MC (1999). Effects of induced parturition and estradiol on feed intake, liver triglyceride concentrations and plasma metabolites of transition dairy cows. J. Dairy Sci., 82:1440-1448.

Bremmer DR, Trower SL, Bertics SJ, Besong SA, BernabucciU,Grummer RR(2000). Etiology of fatty liver in dairy cattle: effect of nutritional and hormonal status on hepatic microsomal triglyceride transfer protein. J Dairy Sci., 83(10): 2239-2251.

Butler ST, Pelton SH, Butler WR (2006). Energy balance, metabolic status, and the first postpartum ovarian follicle wave in cows administered propyleneglycol. J. Dairy Sci., 89: 2938-2951.

Butler WR (2000). Nutritional interactions with reproductive performance in dairy cattle. Anim. Reprod. Sci., 60: 449-457.

Calderon DF, Cook NB (2011). The effect of lameness on the resting behavior and metabolic status of dairy cattle during the transition period in a free stall-housed dairy herd. J Dairy Sci., 94(6): 2883-2894.

Campanile G, Neglia G, DiPalo R (2006). Relationship of body condition score and blood urea and ammonia to pregnancy in Italian Mediterranean buffaloes. Reprod. Nutr., 46: 57-62.

Campanile G, Palo RD, Peretti V, Amante L, Ciotola F, Coletta A (2003). Preliminary results on Mediterranean ItalianBuffalo morphometry. Italian J. Anim. Sci., 2(1): 337-339.

Cardoso FC, Kalscheur KF, Drackley JK (2020). Symposium review: Nutrition strategies for improved health, production, and fertility during the transition period. J. Dairy Sci., 103: 5684–5693.

Curtis CR, Erb HN, Sniffen LJ (1983). Association of parturient hypocalcemia witheight periparturient disorders in Holstein cows. J. Am. Vet. Med. Assoc., 183:559-561

Dann HM, Morin DE, Bollero GA, Murphy MR, Drackley JK (2005). Prepartum intake, post-partum induction of ketosis and periparturient disorders affects the metabolic status of dairy cows. J Dairy Sci., 88: 3249-3264.

Elitok B, Kabu M, Elitok ÖM (2006): Evaluation of liver function tests in cows during periparturient period. Fırat Univ. Vet.J. Health Sci., 20: 205-209.

Farag EA, Metwally MM (2012). Markers used for prediction of ketosis and milk fever in dairy buffaloes at Kaliobeia governorate. Global Veterinaria, 9: 1-7.

Ferguson SD, Galligan DT (1993). Reproductive programs in dairy

herds. Proc. Centr. Vet. Conference, 161-178, Kansas City, MO.

Fiore E, Giambelluca S, Morgante M, Contiero B, Mazzotta E, Vecchio D, Vazzana I, Rossi P, Arfuso F, Piccione G (2017). Changes in some blood parameters, milk composition and yield of buffaloes (Bubalus bubalis) during the transition period. Anim. Sci. J., 88: 2025-2032

Fleischer P, Metzner M, Beyerbach M, Hoedemaker M, Klee W (2001). The relationship between milk yield and the incidence of some diseases in dairy cows. J. Dairy Sci., 84:2025–2035.

Ghanem MM, El-Deeb WM (2010). Lecithin cholesterol acyltransferase (LCAT) activity as a predictor for ketosis and parturient haemoglobinuria in Egyptian water buffaloes. Res. Vet. Sci., 88(1): 20-25.

GOVS, general organization of veterinary services (2005). Technical veterinary report. General Organization of Veterinary Service, Cairo, Egypt.

Gowda GAN, Zhang, S, Gu H, Asiago V, Shanaiah N, Raftery D (2008). Metabolomics-based methods for early disease diagnostics: A review. Expert. Rev. Mol. Diagn., 8:317-633.

Hagawane SD, Shinde SB, Rajguru DN (2009). Hematological and blood biochemical profile in lactating buffaloes in and around Parbhani city. Vet World, 2:467-469.

Hammon DS, Evjen IM, Dhiman TR, Goff JP, Walters JL (2006). Neutrophil function and energy status in Holstein cows with uterine health disorders. Vet. Immunol. Immunopathol., 113: 21-29.

Hassaneen A, Ramadan M, Zain-Elabedeen A, Zeidan A, Ahmed AE, Rawy MS, Kitahara G, Osawa T (2020). Ketosis-Related Biochemical Changes in Crossbred (Tarentaise X Egyptian Baladi) Dual-Purpose Cows and its Effect on the Subsequent Fertility. 163rd Meeting of Jap. Soc. Vet. Sci., 163: 283.

Herdt TH (2010). Ruminant adaptation to negative energy balance. Influences on the etiology of ketosis and fatty liver. Vet. Clin. North. Am. Food Anim. Pract., 16(2): 215-230.

Huber TL, Wilson RC, Stattelman AJ, Goetsch DD (1981). Effect of hypocalcemia on motility of the ruminant stomach. Am. J. Vet. Res., 42:1488.

Ingvarsten KL, Andersen JB (2000). Integration of metabolism and intake regulation: a review focusing on periparturient animals. J. Dairy Sci., 83(7):1573-1597.

Kessel S, Stroehl M, Meyer HHD, Hiss S, Sauerwein H, Schwarz FJ, Bruckmaier RM (2008). Individual variability in physiological adaptation to metabolic stress during early lactation in dairy cows kept under equal conditions. J. Anim. Sci., 86:2903–2912.doi: 10.2527 /jas.2008-1016.

Khan A, Akhtar MZ (2007). Hemato-biochemical and clinico-epidemiological aspects of parturient haemoglobinuria in Nili-Ravi buffaloes. Ital. J. Anim. Sci., 6(2): 953-956

Konvicna J, Vargova M, Paulikova I, Kovac G, Kostecka Z (2015). Oxidative stress and antioxidant status in dairy cows during prepartal and postpartal periods. Acta Veterinaria Brn., 84(2): 133-140.

LeBlanc S (2010). Monitoring metabolic health of dairy cattle in the transition period. J. Reprod. Devel., 56: 29-35.

Magnus P, Lali KF (2009). Serum biochemical profile of post-partum metritic cow. Vet. World, 2(1): 27-28.

Mashek D, Ingvartsen KL, Andersen JB, Vestergaard M, Larsen T (2001). Effects of a four-day hyperinsulinemic-euglycemic clamp in early and mid-lactation dairy cows on plasma concentrations of metabolites, hormones, and binding proteins. Domest. Anim. Endocrinol., 21:169–185.

Meena VK, Sharma SK, Meena S, Chandolia LK, Jhoshi M, Gaur M, Khinchi RK, Jediya HK (2022). Prevalence of periparturient disorders during transition period in buffaloes. The Pharma Innovation J, 11(8): 407-412.

Miqueo E, Chiarle A, Giuliodori MJ, Relling AE (2019). Association between prepartum metabolic status and resumption of postpartum ovulation in dairy cows. Domest. Anim. Endocrinol., 69: 62–67.

Mohamed GA, Abd-Elnaser EM, Elsayed HK (2015). Preliminary study on lipid profile with relation to total antioxidant capacity and some hematological and biochemical changes of pre-post-partum buffalo heifers at Assiut city. Assiut Vet. Med. J., 61(144):159-165.

Nazifi S, Mohebbi FM, Rowghani E, Behbood MR (2008). Studies on the relationship between subclinical ketosis and liver injuries within the first two months of lactating period in high producing Iranian Holstein cows. Int. J. Dairy Sci.., 3(1): 29-35

Ospina PA, Nydam DV, Stokol T, Overton TR (2010). Associations of elevated non-esterified fatty acids and beta-hydroxybutyrate concentrations with early lactation reproductive performance and milk production in transition dairy cattle in the northeastern United States. J. Dairy Sci., 93:1596-1603.

Overton TR, Waldron MR (2004). Nutritional management of transition dairy cows: strategies to optimize metabolic health. J. Dairy Sci., 87: 105-119

Padilla L,Shibano K, Inoue J,Matsui T, Yano H(2005). Plasma vitamin C concentration is not related to the incidence of ketosis in dairy cows during the early lactation period. J Vet. Med. Sci., 76: 883-886.

Purohit M, Gaur C, Saraswat S, Bihani DK (2013). Metabolic Disorders in the Parturient Buffalo, Purohit G.N. (Ed.). International Veterinary Information Service, Ithaca NY (www.ivis.org), A5721.1013.

Ramadan MH, Mahmoud AZ, Zeidan AEB, Ahmed AE, Hassaneen ASA (2020). Puerperal Metritis in Crossbreed (Tarentaise X Baladi) Cows: Metabolism-Related Biochemical and Haematological changes. J. Adv. Vet. Res., 10(2): 96-104. https://advetresearch.com/index.php/AVR/article/view/444

Risco CA, Drost M, Thatcher WW (1994). Effects of retained fetal membranes, milk fever, uterine prolapse or pyometra on postpartum uterine and ovarian activity in dairy cows. Theriogenology, 42:183.

Roche JR, Friggens NC, Kay JK, Fisher MW, Stafford KJ, Berry DP (2009). Invited review: body condition

score and its association with dairy cow productivity, health, and welfare. J. Dairy Sci., 92: 5769-5801.

Sammad A, Khan MZ, Abbas Z, Hu L, Ullah Q, Wang Y, Zhu H, Wang Y (2022). Major Nutritional Metabolic Alterations Influencing the Reproductive System of Postpartum Dairy Cows. Metabolites, 12: 60.

Seifi HA, Gorji-Dooz M, Mohri M, Dalir-Naghadeh B, Farzaneh N (2007). Variations of energy-related biochemical metabolites during transition period in dairy cows. Comp. Clin. Pathol., 16(4):253-258.

Sharma IJ and Rakesh K (2001). Correlation of some blood biochemicals with ketone bodies in normal and sub-clinical ketotic bovines Indian J. Anim. Sci., 71(11): 1029-1031.

Sheldon IM, Lewis GS, LeBlanc S, Gilbert RO (2006). Defining postpartum uterine disease in cattle. Theriogenology, 65: 1516–1530.

Terzano GN, Allegrini S, Borghese A (2005). Metabolic and hormonal parameters in buffaloes. Chapter XII In: Buffalo Production and Research, ed. Borghese A, FAO REU Technical series, FAO Rome, 219-247.

Tharwat M, Takamizawa A, Hosaka YZ, Endoh D, Oikawa S (2012). Hepatocyteapoptosis in dairy cattle during the transition period. Canadian J. Vet. Res., (4): 241-247.

van Knegsel AT, Hammon HM, Bernabucci U, Bertoni G, Bruckmaier RM, Goselink RM, Gross JJ, Kuhla B, Metges CC, Parmentier HK (2014). Metabolic adaptation during early lactation: Key to cow health, longevity and a sustainable dairy production chain. CAB Rev., 9:15.

Velázquez MML, Peralta MB, Angeli E,Stassi AF, Gareis NC, Durante L, Cainelli S,Salvetti NR, Rey F, Ortega HH (2019).Immune status during postpartum, peri-implantation and early pregnancy in cattle: An updated view. Anim. Reprod. Sci., 206: 1-10.

Vermunt J (2003). A brief review and observations on clinical ketosis in non-lactating dairy cattle. New Zealand Vet., 35: 121-123.

Walsh RB, Walton JS, Kelton DF, LeBlanc SJ, LeslieKE, Duffield TF (2007). The effect of subclinical ketosis in early lactation on reproductive performance of postpartum dairy cows. J. Dairy Sci., 90: 2788-2796.

Wathes DC, Fenwick M, Cheng Z, Bourne N, Llewellyn S, Morris DG, Kenny D, Murphy J, Fitzpatrick R (2007). Influence of negative energy balance on cyclicity and fertility in the high producing dairy cow. Theriogenology, 68: S232–S241

Webster CC, Wilson PN (1980). Agriculture in the Tropics. Second ed. Longman (ELBS), London, UK. 390-400.

Yameogo N, Ouedraaogo GA, Kanyandekwe C, Sawadogo GJ (2007). Relationship between ketosis and dairy cows' blood metabolites in intensive production farms of the periurban area of Daker. Trop.Anim.Heal.Prod., 40(7): 483-490.

Youssef MA, El-Khodery SA, El-Deeb WM (2010). Ketosis in buffalo (Bubalus bubalis): clinical findings and the associated oxidative stress level. Trop. Anim. Health. Prod., 42:1771-1777.

Zhang Y, Li X, Zhang H, Zhao Z, Peng Z, Wang Z, Liu G, Li X (2018). Non-esterified fatty acids over-activate the tlr2/4-nfkb signaling pathway to increase inflammatory cytokine synthesis in neutrophils from ketotic cows. Cell. Physiol. Biochem., 48: 827–837.