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Association among antioxidant status, hormonal profile, and biochemical parameters during the periparturient period of dairy cattle in Upper Egypt

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Abstract. The present study attempted to evaluate the physiological modification in the antioxidant status, hormonal and biochemical profile of dairy cattle in Upper Egypt during the periparturient period. Blood samples were obtained from the jugular vein of 25 healthy dairy cattle from a private dairy farm in Assiut province, Egypt. Blood samples were taken at 7 days' intervals: two weeks before and two weeks after parturition (during the periparturient period). The collected serum samples were analyzed for the determination of biochemical and hormonal parameters, including progesterone (P_4), estradiol (E_2), cortisol, triiodothyronine (T_3), thyroxine (T_4), total antioxidant capacity (TAC), malondialdehyde (MDA), blood glucose (BG), triacylglycerol (TG), Total cholesterol (TC), calcium (Ca^{2+}) and phosphorus (P^{3+}). Our results indicated that serum P_4 levels were significantly decreased post-partum than during the pre-partum period. E_2 level reached the maximum on the day of calving as compared with the pre-partum period. The serum cortisol level was highly elevated at calving day. T_3 concentration was significantly higher on the 7th day post-partum, while a higher level of T_4 was recorded on the 15th-day post-calving. TAC and MDA showed significant changes during our study. The BG increased significantly, reaching the maximum level on the calving day, then it was sharply decreased on the 7th-day post-calving. TG and TC levels showed a significant reduction in two weeks post parturition. A significant reduction of both Ca^{2+} and P^{3+} was noted at the calving and post-partum period. The periparturient period was associated with significant hormonal, oxidative stress, and biochemical blood profiles compared with the pre-partum period.

Keywords: *Dairy cattle, periparturient period, oxidative stress, hormonal disturbance.*

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1. Introduction

A transition period (periparturient period) is the time that extends from late pregnancy (3 weeks before parturition) to early lactation (3 weeks post-calving). It is considered an essential and critical stage to the health, production, and reproduction of dairy cows because most of the metabolic and infectious diseases occur throughout this period [1-3]. It is characterized by remarkable physiological, metabolic, and nutritional modifications, resulting from the changes in the animal's endocrine status to support the late pregnancy, events of parturition, and the start of milk production [4].

After parturition, the energy required for milk synthesis is not covered by the cows' voluntary feed intake, creating a negative energy balance (NEB) for most of them. Overcoming NEB, the mobilization of body reserve from adipose tissue leads to elevated reactive oxygen species (ROS). The decreased dietary intake and increased ROS and result in the oxidative stress status of dairy cows [5].

The stressful condition during the transition period and the decreased food intake with a drop in the concentration of different blood constituents make it very important in the animal's life. Therefore, the present study aims to evaluate these cattle's antioxidant status and metabolic profiles besides, measurement the level of some reproductive and stress hormones during the periparturient period.

2. Materials and methods

2.1. Management, animals, and study design

The study was conducted on a private dairy farm in El-Wasiti Village in Assiut province, Egypt, from January to March 2017. A total of 25 pregnant regular healthy periparturient dairy cattle were used for the present study (2-7 years age, 2-6 parity, 250-410 kg weight with an average daily milk production 15-23 kg). These animals were kept under regular feeding and management conditions for their requirements for pregnancy and the demands of milk production. These cows were monitored for about two weeks before the suspected time of calving until about two weeks after calving. The gynecological and medical examination was performed. After gynecological and medical examination, these selected cows were healthy and normally calved without any placental retention or dystocia and the puerperium period was physiological; also, they were free from any metabolic or uterine disorders.

2.2. Collection of blood samples

Blood samples were collected from the jugular vein of healthy periparturient dairy cattle early in the morning, and all efforts were made to decrease the distress. Samples were collected at the pre-partum period (-15th and -7th days before calving), then at calving day (0 days), and finally at the post-partum period (+7th and +15th days after calving). Blood samples were taken in plain vacutainers and kept in an inclined position for 15 minutes at room temperature. They were put in the refrigerator to avoid glycolysis, and for complete retraction of the blood clot, the samples were centrifuged at 3000 rpm for 10 minutes till the clear serum was separated, which is carefully collected and stored in Eppendorf tubes at –80 ˚C until time of biochemical assessment.

2.3. Serum biochemical analysis

According to the enclosed pamphlet, ELISA Kit determined both T3 and T4 supplied from Pishtaz Teb Diagnostic (Germany). P_4, E_2, and cortisol serum levels were analyzed using ELISA Kit supplied from Diameter (Italy), Bio check (USA), and Diagnostic Biochem, Canada Inc. (Canada), respectively, and all of them were determined according to the enclosed pamphlet.

Total antioxidant capacity (TAC) and (MDA) were investigated by using commercial kits supplied from Biodiagnostic Company (Egypt). The determination of TAC was performed according to the methods of [6]. In comparison, the MDA level was estimated according to the methods described by [7,8].

The Kits of blood glucose (BG), triacylglycerol (TG), and total cholesterol (TC) were obtained from Human (Germany), while calcium (Ca^{+2}) and phosphorus (P^{3+}) kits were obtained from Spectrum Co. (Germany) and determined by quantitative colorimetric method according to kits instruction.

2.4. Statistical analysis

Serum biochemical parameters were analyzed by analysis of variance (ANOVA) to compare the mean of different groups. Multi comparison test (One Way ANOVA: Post Hoc multiple comparison LSD, Duncan) was used to test the significance of studied means using SPSS 23 for the window. The two groups were significantly different if the p-value was statistically lower than 0.05 ($p<0.05$).

3. Results and discussion

3.1. Effect of the periparturient period on serum reproductive hormones

The serum P_4 levels were significantly decreased at calving and post-partum periods than at the prepartum period, where the minimum value of P_4 was (0.62 ± 0.11 ng/ml) on the 15th day after calving (Figure 1). On the other hand, E_2 serum level gradually increased at the prepartum period reaching its maximum level at calving with a subsequent decrease (37.67 ± 3.71 Pg/ml) at +15d post-partum (Figure 2).

The transition period is considered one of the most significant metabolic stressful periods in a dairy cow's life. They undergo massive physiologic and metabolic changes to bring themselves ready for the onset of lactation and rise to the peak of milk production [9].

In the present study, a significant decrease was evident in serum P_4 concentration at both calving and post-partum periods than the pre-partum period; our results are in the same aspect with [10]. Also, In Baladi, cows found that the P_4 level was significantly elevated with the advanced of pregnancy than at the end of the third trimester of pregnancy; it decreased, reaching its lowest values from 6 to 1day pre-partum [11]. Likely, the buffalo found that P_4 reduced gradually over the last 7 days of pregnancy with short falling 1-2 days before parturition [12]. Similarly, [13] declared that P_4 levels decreased to 1.2-2.0ng/ml at the last 12-24 hours' pre-partum then to be less than 1 ng/ml 24-48 hours post-partum in local Egyptian cattle. Nessim [14] cited that this P_4 reduction was physiologically at calving due to the destruction of pregnancy's corpus luteum (CL). At parturition, increase the concentration of Prostaglandin $F_2α$ (PGF_2 α) and causing CL luteolysis and inhibits P_4 synthesis [15].

On the other hand, our result showed a significant increase in serum E_2 level at the pre-partum period with a maximum level at calving day then decreased sharply during the post-partum period. These results were in harmony with Abd-El-Rahman et al. [16], who mentioned that the highest E_2 level was present during the third trimester of gestation in she-camell and increased significantly with pregnancy progress, then E_2 sharply decreased after calving. Also, Purohit [12] noted that in the buffalo, the plasma concentrations of E_2 start increasing 7 days' prepartum to reach maximum levels 1 day before parturition. The same results were obtained in Baladi cows [11] and Friesian cows [17], and E_2 level significantly increased with the advancement of pregnancy, and after calving, E_2 level decreased. The high E_2 concentration in the pre-partum period has an essential role in initiating oxytocin release, which is essential for uterine contractions to aid in fetal expulsion during parturition [18]. EL-Msry et al. [19] demonstrate that the high E_2 and P_4 levels in the third trimester of pregnancy have triggered the normal development and growth of the alveolar duct system mammary gland to get the udder of cows into milking processes.

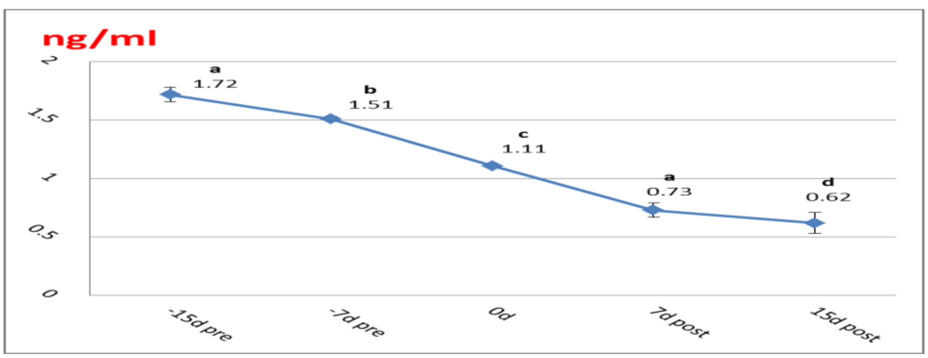

Figure 1. Mean ± SE of serum P_4 concentrations (ng/ml) of dairy cattle during periparturient period (pre = pre-partum, 0d = day of parturition, post = post-partum). Means not followed by the same letter differ significantly (p<0.01).

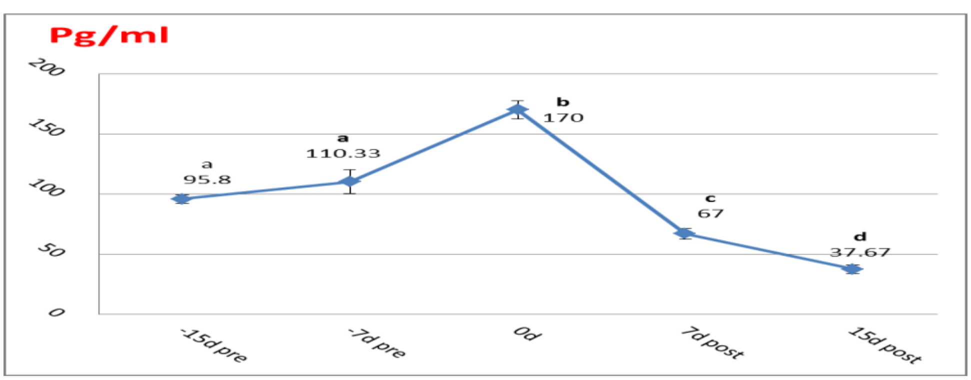

Figure 2. Mean ± SE of serum E2 concentrations (Pg/ml) of dairy cattle during periparturient period (pre = pre-partum, 0d = day of parturition, post = post-partum). Means not followed by the same letter differ significantly (p<0.01).

3.2. Effect of the periparturient period on serum cortisol, T_3 and T_4

The serum cortisol level increased significantly in our findings, reaching its peak serum level (10.32 ± 1.14 µg/dl) at 0 days of calving compared to pre-partum (Figure 3). Cortisol is considered the primary stressful hormone produced by the adrenal glands in the ruminant [20]. Therefore, during this period, the increased cortisol level indicates that these cattle were under stress in late pregnancy, which is ended after parturition [14]. It is evident from the present study that serum cortisol concentration was increased significantly, reaching a maximum level at caving day then decreased. These results were agreed with the findings of [16], who mentioned that in late pregnancy, there is an increase in the fetal Adrenocorticotrophic hormone secretion from the fetal pituitary gland to stimulate the rapid growth and development of the fetal adrenals, leading to an elevation in serum cortisol level. This increased cortisol enters the maternal circulation causing a rise in the maternal serum cortisol level. Fetal cortisol stimulates placental E_2 production [21], which stimulates uterine $PGF_{2}α$ secretion causing CL destruction and interrupts P_4 production [22]. These changes in the hormones of the dams allow the cervical dilation to permit the passage of the fetus through the birth way and allow the uterine contractions, which assist in both fetal and placental expulsion. It is evident from the combination of these hormones between the dam, fetus, and placenta, the significant role in the initiation of parturition in dairy cattle [12]. Additionally, cortisol is essential for the surface formation of the fetal lung to prevent hyaline membrane disorder and respiratory insufficiency [16]. Besides, cortisol is considered an immune-suppressive hormone that diminishes the immunological response against the fetus, therefore protecting the fetal life. Also, cortisol enhances gluconeogenesis that provides sufficient BG supply for fetal nutrition [23].

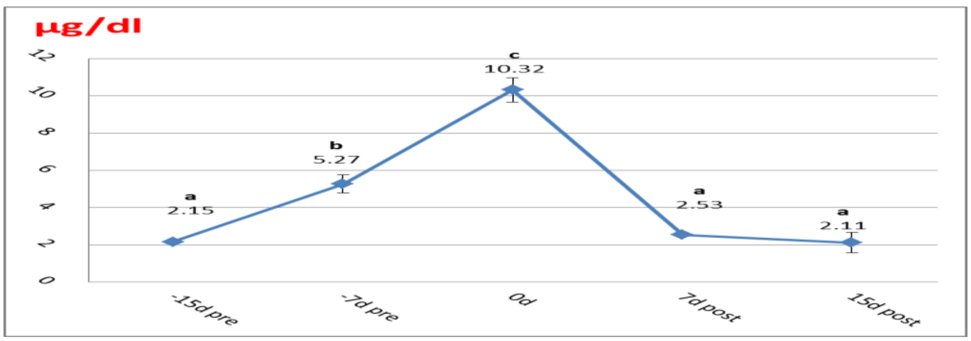

Figure 3. Mean ± SE of serum cortisol concentrations (µg/dl) of dairy cattle during periparturient period, (pre = pre-partum, 0d = day of parturition, post = post-partum). Means not followed by the same letter differ significantly (p <0.001).

Higher Plasma T_3 level was significantly obtained at +7d post-partum (2.72 ± 0.04 ng/ml) compared with the pre-partum period and at caving day (Figure 4). However, the T_4 serum level was significantly elevated (5.66 ± 0.09 µg/dl) at +15d post-partum compared to prepartum period and calving day (Figure 5).

Thyroid hormones have an essential role in adapting the endocrine system during the transitional period [24]. The present study revealed a significant increase in serum level of T_3 in the post-partum period compared to pre-partum, which correspond to [14,25]. This increase resulted from the rise in metabolic rate to rebuild the destructed tissues and compensate for the blood metabolites deficiency during pregnancy [16]. Also, Fiore et al. [1] declared that NEB at late pregnancy, besides the high utilization of these hormones by the mammary gland, may be responsible for reducing serum T_3 and T_4. E_2 can alter the rate of thyroid hormone secretions and its dynamics; it seems that alterations in thyroid activity may be related to the interactions with the variable concentrations of E_2 and P_4 during late pregnancy [16,26].

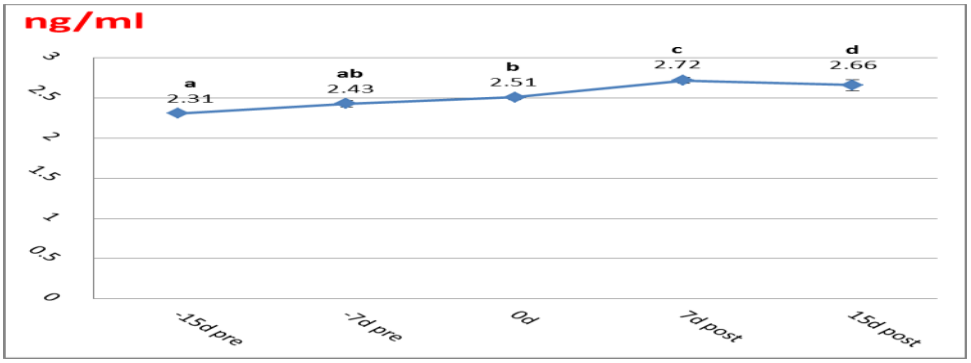

Figure 4. Mean ± SE of serum T_3 concentrations (ng/ml) of dairy cattle during periparturient period (pre = pre-partum, 0d = day of parturition, post = post-partum). Means not followed by the same letter differ significantly (*p*<0.05).

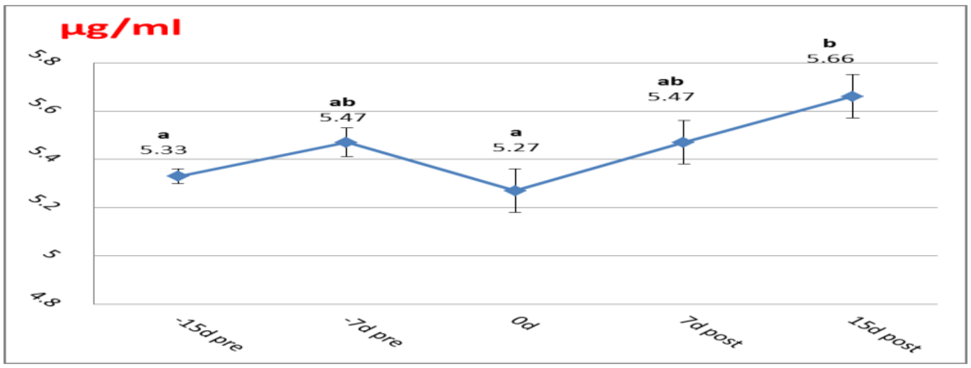

Figure 5. Mean ± SE of serum T_4 concentrations (µg/ml) of dairy cattle during periparturient period (pre = pre-partum, 0d = day of parturition, post = post-partum). Means not followed by the same letter differ significantly (*p*<0.01).

3.3. Effect of the periparturient period on serum oxidative

Changes in the results of both TAC and MDA serum levels during the periparturient period in dairy cattle are presented (Figure 6). TAC concentration decreased significantly at calving than the prepartum period then, a significantly higher value (1.98 ± 0.06 mM/L) was obtained at +15d post-calving. On the other hand, serum MDA was significantly elevated (11.83 ± 0.71 nmol/ml) at +7d post-partum compared with the pre-partum period. Our study indicated that dairy cows were exposed to an increased risk of oxidative stress during the periparturient period, as suggested by the observed TAC decreased levels and increased MDA concentrations. The lower mean value of TAC at the pre-partum period and calving as demonstrated in our study was similar to the results of [27-29]; they cited that such reduction may result from insufficient vitamins and mineral supplement stress parturition with increased demands for lactation. In the same line, [30] mentioned that TAC concentration was significantly lower before calving, and the highest TAC level was observed 8 weeks post-partum [31].

It is considered that MDA is a product of lipid peroxidation and a marker of oxidative stress [32]. In this work, the MDA level was significantly higher in post-partum dairy cattle, which conforms with the results of [9], who mentioned that the highest MDA level in dairy cows was recorded in the 1st week after parturition. In the same trend, [33] recorded a transient elevation of MDA levels in dairy cows in this period after parturition. Moreover, [34,35] also noted an increased MDA concentration in periparturient cows**.** This significantly increased MDA levels may have related to excessive lipolysis and fatty acids mobilization to tolerate the high milk production. Moreover, NEB during the post-partum period may cause the acceleration of fat mobilization and oxidation of fatty acids [10]. NEB during late pregnancy and early lactation period is usually accompanied by oxidative stress [36]. During the NEB, non-essential fatty acids (NEFA) enter into the mitochondria of hepatocytes, oxidized to produce energy. Thus, the increased NEFA oxidation produces a large amount of ROS, resulting in elevated lipoperoxidation mechanism subsequently, changes in the pro-oxidative/anti-oxidative status [37]. Also, [38] observed higher MDA in the plasma during the early lactation period suggest that in the 1st weeks after parturition, the body presents high amounts of free radicals, which cause excessive lipid peroxidation; this effect is related to the intensity of the metabolic changes.

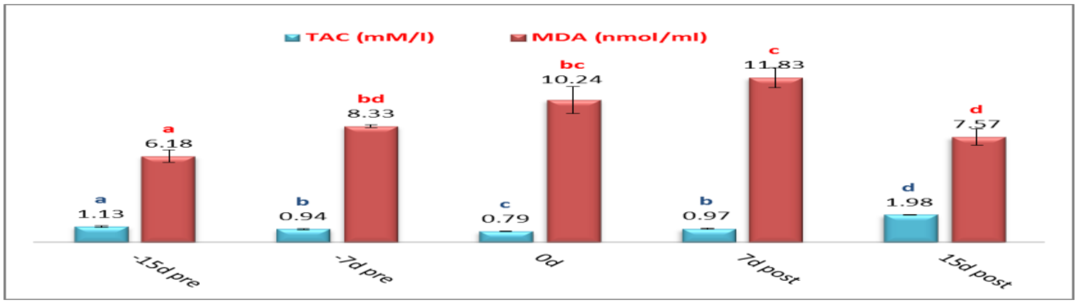

Figure 6. Mean ± SE of serum TAC (mM/L) and MDA (nmol/ml) concentrations of dairy cattle during peripaturient period (pre = pre-partum, 0d = day of parturition, post = post-partum). Means not followed by the same letter differ significantly (p<0.05).

3.4. Effect of the periparturient period on serum BG, TG, and TC

Data indicated that the serum glucose increased significantly at caving day (49.90 ± 2.05 mg/dl) compared to a pre-partum period followed by a significant decrease at +7 d post-calving (36.90 ± 1.49 mg/dl). As for TG and TC, our results revealed a significant reduction at calving day and continued to decrease, reaching its minimum level at +15 d post-partum (Figure 7) significantly.

Glucose is the primary metabolic fuel and is needed for the function of a vital organ, fetal development, and milk production [39]**;** also, BG is considered as an energy source for ovarian function, and the lowered BG level at early lactation harms post-partum resumption of ovarian activity [40]. Our results indicate that the higher level of BG at calving day was in agreement with [4]. This hyperglycemia may be associated with the stress of calving, and the increased cortisol and E_2 level enhance the glycogenesis [41,42]. Although in our results, we noted that the BG concentration was significantly lowered in 1st week at early post-partum than in the pre-partum period, then it increased after that, these findings were coincided [43]**,** who found that the start of lactation was associated with a reduction in BG. Also, [44,45] found that BG concentration was abruptly reduced after parturition and then followed by a slight rise after that. Herdt [46] attributes that BG reduction to the low-energy balance in the early lactation period. However, the lowered glucose value at +7d post-partum may be due to the NEB in the early lactating cows and the excessive utilization of BG by the mammary gland to produce lactose hypoglycemia and excessive mobilization of body reserves to support additional energy [10].

Our data concerning TG are coinciding with those obtained by [31,47]**,** who noticed a significant decrease in TG value at the post-partum period and in the early lactating period than at the prepartum and calving day. The reasons for such reduction may be related to the transfer of TG for the synthesis of milk fat components in the mammary gland [4,31] and TG precipitation in the liver [48,49].

The reduction in TC level at the calving and post-partum period in our study corresponds to the finding of [42]. Before parturition, the lowered TC values may be returned to its consumption by the ovaries and placenta for steroidogenesis [50]. Moreover, [51] described that reduced TC began from parturition and post-calving weeks, reducing dry matter intake**.** Also, the reduction in serum TC concentration during late pregnancy is may due to the increased utilization of fetal tissues and maternal glands for steroid hormone synthesis [52].

Contrariwise, [53] suggested that the immunological status of dairy cows after parturition is highly related to serum TC levels during the dry period. This suggestion is also reported by [53,54], they found that the levels of some haematochemical parameters liver product can be used as an indicator for the physiological stress, this can indicate that the parturition and the post-partum periods consider as the most stressful phases during the productive life in the dairy cows.

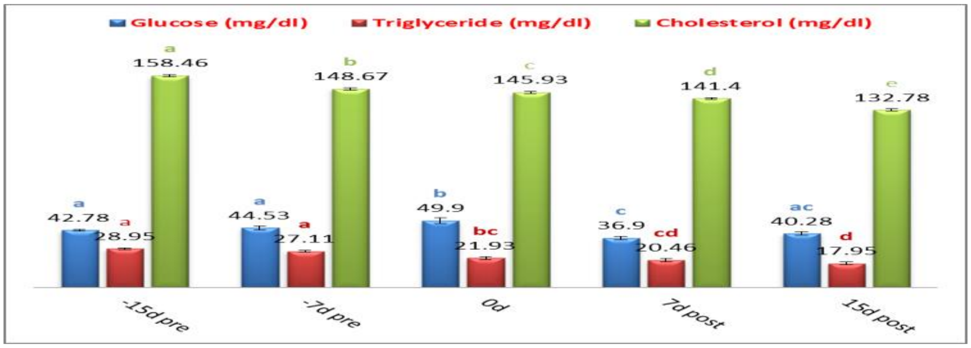

Figure 7. Mean ± SE of blood metabolites concentrations of dairy cattle during periparturient period (pre = pre-partum, 0d = day of parturition, post = post-partum). Means not followed by the same letter differ significantly (*p*<0.05).

3.5. Effect of the periparturient period on serum Ca2+ and P3+

Our results revealed a significant reduction trend of both elements at calving and post-partum as compared with the pre-partum period (Figure 8). Minerals are essential nutrients that play a significant role in reproduction because an increase or decrease in their levels has a notable effect on the animals' performance [55]. Additionally, minerals are required for the development of the mammary gland, the growth of the fetus, and support the function of the immune system [56]. The lowered Ca^{2+} and P^{3+} level at the post-partum period, as denoted in the present study, was in the same aspect as that observed by [57,58], who demonstrated that their deficiency may be related to inadequate absorption of food metabolite from the gastrointestinal tract, inadequate Ca^{2+} bone mobilization and increased its excretion in urine. Moreover, drainage of a high amount of Ca^{2+} and P^{3+} in colostrum during excessive lactation, mainly after parturition [27]. The periparturient dairy cows have an abrupt increase in Ca^{2+} demand for fetal growth, lactation, and reproduction [59]. Chamberlin et al. [60] cited that most hypocalcaemia events in dairy cows occur mainly in the first 24 hours following parturition. Milk P^{3+} and Ca^{2+} output are instantly related to milk yield, as milk P^{3+} level is stable [61]. Besides, [62,63] declared that the reduced P^{3+} level might attribute to the inadequate P^{3+} supplement in the diet and increased P^{3+} excretion in urine due to hyperparathyroidism; this could explain this post-partum hypophosphatemia.

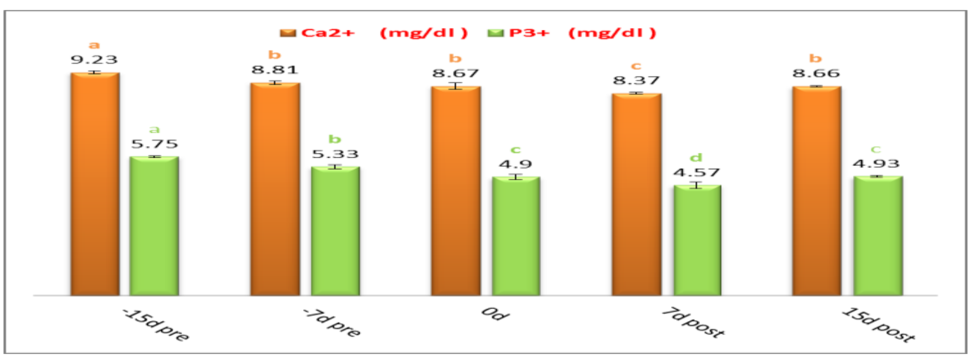

Figure 8. Mean ± SE of serum minerals concentrations of dairy cattle during periparturient period (pre = pre-partum, 0d = day of parturition, post = post-partum). Means not followed by the same letter differ significantly (*p*<0.05).

Conclusion

Our presented study indicates significant changes in the values of hormonal as progesterone, estradiol, and cortisol during the periparturient period were helpful to detect the prediction time of parturition in dairy cattle. Also, dairy cattle during this period were under stress, making them more susceptible to many metabolic disorders. So, hormonal and metabolic modification with alteration in the immune mechanism was carried out to pass this critical period without any pathological disorder successfully.

Conflicts of interest. There is no conflict of interest.

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