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Effects of vitamin D administration at the beginning of lactation in dairy cows on inflammatory response and liver metabolism

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Abstract: This study aimed to investigate the effect of vitamin D administered 24 h after calving of dairy cows on calcium-phosphorus metabolism, carbohydrate, lipid and liver metabolism, and inflammatory response. Twenty multiparous Holstein cows were randomly assigned to the experimental (EG) and control group (CG). The EG received 5,000,000 IU vitamin D_3 (5 mL) 24 h after parturition intramuscularly, whereas the CG group received 5 mL of 0.9% NaCl. Blood samples were taken 24 h after parturition before treatments and at the 3rd, 7th, 14th, and 28th days of lactation. Serum NEFA, BHBA, haptoglobin, serum amyloid A (SAA), ceruloplasmin, calcium, phosphorus, parathormone, total protein, triglyceride, AST, and GGT were measured. In addition, total bilirubin, albumin, total cholesterol, and vitamin A concentrations were also measured to calculate liver activity index and the liver functionality index. Subclinical hypocalcemia (SCH) was observed in only one cow in the EG and five in the CG after the 3rd day postpartum. NEFA levels in the EG significantly differed on days 7, 14, and 28 when compared to vitamin D pretreatment values. However, NEFA levels in the CG differed only between day 28 postpartum and the 1st day ($p < 0.05$). Negative correlation was determined between vitamin D and NEFA ($p < 0.01$). Conversely, negative correlations were observed between calcium and NEFA, haptoglobin, SAA ($p < 0.05$). In conclusion, it can be suggested that vitamin D administration can prevent SCH after the 3rd day and has limited positive effects on postpartum NEFA levels.

Key words: Dairy cow, vitamin D, calcium, NEFA, liver metabolism, inflammation

1. Introduction

In dairy cows, the critical period starting from 3 weeks before giving birth and continuing until 3 weeks after giving birth is called the transition period [1]. When not well managed, due to the negative effects of calving stress and high milk yield, many problems are observed in dairy cows during this period [1]. Dairy cows in the postpartum period lose phosphorus and calcium due to colostrum production at the beginning of lactation and the gradual increasing milk yield. Although clinical hypocalcemia occurs uncommonly in dairy cows, [2] subclinical hypocalcemia (SCH) is commonly seen, and it causes many problems that include depressed immune functions, ketosis, fatty liver, and displacement of the abomasum [3].

One of the most affected systems after parturition in cows is the immune system [4-6]. The first response of the immune system to harmful stimuli is local inflammation. When the infection and tissue damage exceed regional defense, the organism produces a broader and detailed systemic response. This reaction is generally described as the acute phase response. While the inflammatory process initiates, the production of positive acute-phase protein (APP) is increased and the production of negative APP production is decreased [7]. Generally, these proteins are produced mostly in the liver, but they are also produced in less significant amounts in peripheral tissues too. In ruminants, haptoglobin (Hp) and serum amyloid A (SAA) are considered to be the most prominent positive APP, and albumin and cholesterol are considered negative APP [8,9].

Inflammation is not only significant in clinical diseases but also significant in subclinical diseases. Subclinical effects caused by inflammation are decreased appetite and enhanced negative energy balance and lipolysis. It is suggested that all these effects increase the prevalence of ketosis, fatty liver disease, and displacement of the abomasum in the postpartum period [4]. The liver activity index (LAI) and liver functionality index (LFI) use negative APP in blood [4].

Vitamin D, which provides the absorption of calcium and phosphorus from intestines, plays an extremely important role in calcium and phosphorus metabolism [10]. In recent years, many studies were conducted on vitamin D and its regulatory effects on the immune system

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and inflammatory process besides its effects on calcium and phosphorus mechanism [11,12].

We hypothesized that vitamin D may improve liver functions and alleviate negative energy balance in dairy cows, immunoregulatory and antiinflammatory effects in the postpartum period. The objective of this study was to investigate the effect of vitamin D administered 24 h after calving besides calcium-phosphorus metabolism, carbohydrate and lipid metabolism, and particularly the effects on inflammatory parameters and liver functions in dairy cows.

2. Materials and methods

2.1. Ethical approval

The experimental procedures were approved by the Committee of Animal Experiments of Bursa Uludağ University. Approval number: 2017 – 10 / 03

2.2. Animals and management

Twenty multiparous Holstein dairy cows were randomly divided into two equal groups as experimental (EG) and control group (CG), between June and December 2018. The cows enrolled in the study were 2.8 ± 0.4 and 3.0 ± 0.3 lactation numbers in the EG and CG, respectively. The mean condition scores were determined as 2.72 ± 0.1 and 2.83 ± 0.1 in the EG and CG, respectively. EG received 5,000,000 IU (International Unit) vitamin D_3 (EGEVET-D3 inj., İzmir-Turkey) (5 mL) 24 h after parturition intramuscularly. Half of the 10 million IU vitamin D_3 doses were administered prepartum [13] for the prevention of milk fever in cows. Five milliliters of 0.9% NaCl was administered to the CG 24 h after parturition intramuscularly. Blood samples were taken 24 h after calving before vitamin D and placebo administration, and after feeding, at approximately 10.30 am on the 3rd, 7th, 14th, and 28th days postpartum. Cows that had experienced dystocia, mastitis, metritis, or any clinical diseases, etc. for 4 weeks were excluded from the study. Besides, all cows were negative for tuberculosis and paratuberculosis. The cows in both groups were fed the same ration in the prepostpartum period, and they were not fed anionic ration in the dry period. Ingredient and composition of diet were shown in Table 1.

Table 1. Ingredient composition of the diets (as dry matter basis).

Item	Ingredient
Corn silage %	50.81
Alfalfa silage %	13.73
Oat hay %	4.12
Steam flaked corn %	10.99
Soybean meal % Commercial milk feed composition	9.16 11.19
Crude protein %	19
Crude fiber %	6.5
Crude oil %	5
Crude ash % Total %	7 100
Chemical composition %	dry matter
Dry matter %	58.6
Crude protein %	17.03
Neutral detergent fiber %	32.78
Acid detergent fiber %	20.76
Crude ash	7.84

2.3. Blood samples and biochemical analysis

Blood samples were collected and after centrifugation at 3000 rpm for 5 min, serum samples were immediately separated and stored at –20 °C until analysis. Calcium, phosphorus, magnesium, potassium, cholesterol, triglycerides, albumin, total protein, aspartate amino-transferase (AST), gamma glutamyl-transferase (GGT), total bilirubin, and parathormone (PTH) concentrations were determined using Beckman-Coulter commercial kits with Beckman-Coulter AU-680 autoanalyzer. Nonesterified fatty acid (NEFA) and beta-hydroxybutyric acid (BHBA) were analyzed using Randox commercial kits with Biotecnica Instruments BT3500 via the spectrophotometric method [14]. Hp and SAA serum concentrations were measured colorimetrically and by the sandwich ELISA method respectively, and both analyses were performed using bovine-specific commercial kits (Tridelta-Ireland). Ceruloplasmin (Cp) concentrations were measured using the nephelometric method using Siemens BN II commercial kits in the autoanalyzer. Serum concentrations of vitamins A and D were measured with the LC/MS-MS method [15].

2.4. Calculation of LAI and LFI

LAI and LFI were calculated according to the instructions [4]. While calculating LAI; the 7th, 14th, and 28th days concentrations of serum albumin, cholesterol, and vitamin A were used. For calculating LFI, the 3rd and 28th days concentrations of serum albumin, cholesterol, and total bilirubin were used.

2.5. Milk yielding

Milk production of all cows for 30 days was calculated.

2.6. Statistical analysis

All statistical analyses were performed using the SPSS 20.0 program. Normality analysis of data was performed with the Shapiro–Wilk normality test. When examining the differences between groups belonging to the same sampling time, a t-test was used for parameters showing

normal distribution; the Mann–Whitney U test was used for nonnormal distribution parameters. While examining the variation of a parameter in different sampling times within the same group, the parameters showing normal distribution as a result of the Shapiro–Wilk test were found to be one-way ANOVA (Analysis of Variance); the Kruskal–Wallis test was used for parameters not showing normal distribution. Correlation analysis of biochemical parameters was performed using the Pearson correlation test. Additionally, a comparison of LAI and LFI in all cows in both groups was performed with a t-test. The results of the analysis were accepted as significant if $p < 0.05$.

3. Results

Biochemical parameters, APP analysis results of blood samples and correlation coefficients of parameters are described in Tables 2–4, respectively.

According to the calcium levels that were measured at five different sampling times, the SCH rate was determined as 33% (6 cows) in 20 cows and as 41.25% (33 cows) in the first 14 days. Although there are no statistical differences in calcium levels between the two groups; in the EG, the number of subclinical hypocalcemic cows was (serum calcium concentration < 8.00 mg/dL) 7 on the1st day, 8 on the 3rd day, and 1 on the 7th day. However, the number

Table 2. Comparison of biochemical parameters at different times in experimental and control groups.

Parameters	Gr	1st day in milk	3rd day in milk	7th day in milk	14th day in milk	28th day in milk
Calcium (mg/dL)	EG	7.58±0.18^{a}	7.67±0.17^{a}	8.53±0.15^{b}	8.80±0.12^{b}	8.82±0.10^{b}
	CG	7.40±0.20^{a}	7.88±0.17^{ab}	8.18±0.17^{ab}	8.55±0.18^{b}	8.68±0.20^{b}
Phophorus (mmol/L)	EG	1.75±0.11	1.73±0.13	1.53± 0.11	1.43±0.05*^{A}	1.68±0.11
	CG	1.78±0.15	1.82±0.17	1.61±0.09	1.78±0.10*^{B}	1.76±0.11
Parathormone (pg/mL)	EG	312.99±116.63^{a}	114.70±38.80^{b}	56.98±22.46^{cd}	41.71±9.36^{cd}	134.48±78.05^{bcd}
	CG	129.22±29.64^{a}	197.60±10.40^{a}	59.02±11.81^{a}	66.93±22.14^{a}	61.71±22.52^{a}
NEFA (mEq/L)	EG	0.75± 0.13^{a}	0.59±0.09^{ab}	0.43±0.06^{bc}	0.34±0.05^{cd}	0.27±0.04^{d}
	CG	0.97±0.16^{a}	0.81±0.16^{ab}	0.75± 0.15^{abc}	0.61±0.16^{abcd}	0.26±0.05^{e}
BHBA (mmol/L)	EG	0.73±0.08	1.07±0.19	1.10±0.31	0.92± 0.24	0.86± 0.27
	CG	0.65±0.05	0.82±0.09	0.73±0.11	0.66±0.10	0.49±0.04
Albumin (g/dL)	EG	3.29±0.05	3.18±0.08	3.13±0.12	3.18±0.11	3.28±0.07
	CG	3.25±0.04	3.22±0.09	3.17±0.08	3.17±0.10	3.26±0.07
Total Cholesterol (mg/dL)	EG	84.80±5.53^{a}	85.0±6.29^{a}	83.10±8.77^{a}	99.20±10.70^{a}	137.10±10.96^{b}
	CG	77.77±4.41^{a}	77.80±4.76^{a}	78.44±4.77^{a}	88.50±6.62^{a}	115.50±8.16^{b}
Total Bilirubin (µmol/L)	EG	8.37± 1.19^{a}	6.49± 0.85^{a}	6.49± 1.88^{a}	4.95±0.85$^{a\,b}$	3.24±0.17^{b}
	CG	8.03± 1.19^{a}	7.69±2.05^{a}	12.14±4.27^{a}	4.61±0.51$^{a\,b}$	3.07±0.17^{b}
Magnesium (mg/dL)	EG	2.34±0.07^{ac}	7.69±0.13^{ab}	2.07±0.08^{bd}	2.20±0.11^{ad}	2.45±0.06^{c}
	CG	2.51±0.07^{a}	2.62±0.47^{ab}	1.91±0.13^{b}	2.20±0.06^{b}	2.46±0.05^{a}
Potassium (mmol/L)	EG	4.52±0.18	4.52±0.30	4.35±0.15	4.10±0.09	4.14±0.11
	CG	4.67±0.15	4.34±0.12	4.10±0.09	4.28±0.16	4.21±0.18
Triglycerides (mmol/L)	EG	0.18±0.03	0.13±0.0	0.13±0.01	0.14±0.01	0.16±0.01
	CG	0.14±1.78	0.15±0.02	0.13±0.01	0.02±0.02	0.15±0.02
AST (IU/L)	EG	84.30±3.52^{a}	98.10±4.35^{bc}	124.70±15.3^{bc}	105.30±12.59^{ac}	81.10±3.79^{a}
	CG	92.80±11.90^{a}	115.40±18.82^{b}	109.90±12.7^{b}	100.70±8.94^{b}	80.10±3.04^{a}
GGT (IU/L)	EG	23.20±2.40	22.40±2.02	23.50±3.06	27.20±2.58	29.80±3.25
	CG	21.40±1.71	22.20±1.67	24.30±2.45	24.90±1.95	25.90±1.17
Total protein (g/dL)	EG	6.72±0.13^{a}	6.75±0.48^{a}	7.00±0.20^{a}	7.55±0.16*^{bA}	8.12±0.58^{c}
	CG	7.02±0.20	7.24±0.57	7.54±0.18	8.09±0.20*^{B}	8.57±0.34

EG: Experimental Group, CG: Control Group, NEFA: Nonesterified Fatty Acid, BHBA: Betahdroxybutiric Acid, AST: Aspartat Amino Transferase, GGT: Gamma Glutamil Transferase *$P \leq 0.05$. There is no difference between days with the same letter. There are differences between the two groups in columns A, B.

Table 3. Comparison of vitamin A and D and acute phase proteins at different times in experimental and control groups.

Parameters	Gr	1st day in milk	3rd day in milk	7th day in milk	14th day in milk	28th day in milk
Vitamin D (ng/mL)	EG	35.75±3.63^{a}	35.16±3.31^{ab}	40.09±3.17^{*abcA}	52.43±2.97^{*cA}	53.33±3.40^{*cA}
	CG	29.31±1.42^{a}	30.26±1.32^{a}	30.11±1.65^{*aB}	32.66±2.16^{*aB}	36.37±2.94^{*aB}
Vitamin A (µg/dL)	EG	8.46±1.35^{a}	10.96±1.74^{a}	17.33±3.19^{ac}	24.07±4.04^{bc}	30.86±3.19^{b}
	CG	8.72±0.76^{a}	11.22±1.51^{ab}	12.78±2.36^{ab}	17.10±2.33^{b}	26.03±1.56^{c}
Hp (gr/L)	EG	0.70±0.4^{ac}	0.95±0.20^{a}	0.41±0.2^{b}	0.34±0.13^{cb}	0.16±0.06^{b}
	CG	0.69±0.5^{a}	0.69±0.18^{a}	0.61±0.23^{ab}	0.14±0.05^{ab}	0.13±0.03^{a}
SAA (mg/mL)	EG	129±8.35^{a}	124±7.89$^{*A\ a}$	76±17.24^{b}	73±19.50^{b}	47±14.57^{b}
	CG	116±11.4^{a}	90±13.7$^{*\ B\ ac}$	66±17.32^{bcd}	60±16.93^{bc}	44±17.31^{bd}
Cp (mg/dL)	EG	0.39±0.04	0.33±0.04	0.45±0.04	0.42±0.03	0.41±0.01
	CG	0.32±0.04	0.39±0.02	0.43±0.02	0.42±0.03	0.40±0.02

EG: Experimental Group, CG: Control Group, *P ≤ 0.05. There is no difference between days with the same letter. There are differences between the two groups in columns A, B. Cp: Ceruloplasmin, Hp: Haptoglobin, SAA: Serum amyloid A.

Table 4. Correlation coefficients between some parameters.

Parameters	Calcium	NEFA	Vitamin D	Total Cholesterol	Total Bilirubin	Vitamin A	Hp	SAA
Calcium	1	–0.864**	0.678*	0.675*	–0.613	.905**	–0.875**	–0.900**
NEFA	–0.864**	1	–0.782**	–0.800**	.756*	–0.908**	0.694*	0.672*
Vitamin D	0.678*	–0.782**	1	0.742*	–0.594	0.787**	–0.487	–0.389
Total Cholesterol	0.675*	–0.800**	0.742*	1	–0.752*	0.909**	–0.686*	–0.627
Total Bilirubin	–0.613	0.756*	–0.594	–0.752*	1	–0.760*	0.682*	0.468
Vitamin A	0.905**	–0.908**	0.787**	0.909**	–0.760*	1	–834**	–0.825**
Hp	–0.875**	0.694*	–0.487	–0.686*	0.682*	–0.834**	1	0.878**
SAA	–0.900**	0.672*	–0.389	–0.627	0.468	–0.825**	0.878**	1

Table 4 presented important correlations between some parameters. *P ≤ 0.05 **P ≤ 0.001 There are no important correlations determined between the other parameters. NEFA: Nonesterified fatty acid, Hp: Haptoglobin, SAA: Serum amyloid A.

of subclinical hypocalcemic cows was 9 on the 1st day, 5 on the 3rd day, 3 on the 7th day, and 2 on the 14th day in the CG.

Correlations between some parameters are described in Table 4.

In the EG, after vitamin D administration, 2 cows 3 times had NEFA levels > 0.700 mEq/L; on the 3rd, 7th, and 14th days (10%, in 3 measurements, in 10 cows); it was determined that in the CG, 5 cows 13 times (43.33%, in 3 measurements, in 10 cows) had > 0.700 mEq/L NEFA levels. When NEFA levels were compared based on sampling time levels between the groups, a significant difference was found between the 1st and the 7th, 14th, and 28th days in the EG ($p < 0.05$); in the CG, a statistically significant difference was detected only between the 1st and 28th days.

The EG and CG's mean daily milk production was found to be 33.2 ± 3.9 L and 34.06 ± 2.87 L. LAI scores were 0.001 and –0.07 and LFI scores were –2.76 and –3.79, respectively. There was no statistically significant difference between the two group's milk production, LAI and LFI scores.

4. Discussion

In high-yielding dairy cows, low blood calcium level is one of the most significant problems. Generally, it's considered hypocalcemia when the blood serum calcium level is between 6 and 8 mg/dL [2]. In this study, mean calcium levels were determined to be under 8 mg/dL on the 1st and 3rd days in both groups; therefore, the groups are considered to be suffering from SCH [16]. In our

study, the SCH rate was found to be 33%; therefore, the SCH incidence was lower in our study when compared to the previously mentioned one. In another study [17] performed in postpartum dairy cows, blood samples were collected 12–14 h postcalving and on the 4th and 8th day of lactation, and at the end of the study, the SCH rate was determined as 45%. The SCH rate was calculated at 41.25% for the first 14 days of the study. This rate is more parallel with the results of the presented study [17]. In the CG on the 3rd day 5 cows, on the 7th day 3 cows, and on the 14th day, 2 cows were detected to be suffering from SCH, whereas in the EG, although SCH was detected in 8 cows on the 3rd day, it was detected in only one cow on the 7th day. According to these results, although there is no statistically significant difference has been found between the groups, it can be suggested that the administration of vitamin D was more successful in the EG on the 7th day in the prevention of SCH. On the other hand, the probable cause of the higher number of cows with SCH in the EG on day 3 when compared to the CG could be the suppression of PTH as a result of vitamin D administration on the 1st day in the EG [18]. Parallel with this, PTH level decreased significantly ($p < 0.05$) on the 3rd day when compared to the level before vitamin D administration. On the other hand, it was suggested that lower doses of vitamin D (500,000 to 1 million units) may induce milk fever because the high levels of 25-OH vitamin D resulting from the treatment suppress PTH secretion and directly suppress the renal synthesis of endogenous $1,25(OH)_2D$ [11]. However, although 5 million units of vitamin D were used in the EG, milk fever was not observed in this study.

Together with many infectious diseases like mastitis and metritis, physiological problems such as ruminal and intestinal hypomotility leading to decreased appetite and increased NEFA and BHBA levels are observed in dairy cows with SCH [3,19,20]. In a study performed in dairy cows with fatty liver disease, while calcium, total protein, and total cholesterol were higher in the control group than the test group, the NEFA level was lower in the control group [21]. As stated in previous reports, in our study, while we found higher calcium levels in the EG, NEFA levels of the EG were lower than the CG. In contrast to our study, calcitriol was administered to dairy cows 6 h after parturition, and animals were observed for 15 days [18]. When the levels of NEFA in both groups were compared, the NEFA of the CG was lower than the EG. However, there was no statistically significant difference between the groups, similar to our study.

Although there was no significant difference between NEFA levels of the groups on day 1, it was noted that NEFA levels were higher in the CG than EG, especially on the 3rd, 7th, and 14th days. It is important to note the difference in the number of the cows that are above the threshold was NEFA > 0.700 mEq/L, [22]. When NEFA levels were compared within the groups, a significant difference was found between the 1st and 7th, 14th, 28th days in the EG ($p < 0.05$); in the CG, a statistically significant difference was detected only between the 1st and 28th days. In addition to this, increased levels of calcium not only decreased NEFA release from peripheral adipose tissues but there was also a positive and strong correlation between calcium and total protein, total cholesterol, vitamin D ($p < 0.05$), and vitamin A ($p < 0.01$).

In this study, the difference in BHBA levels was not significant between the two groups. BHBA levels were contradictive with the NEFA results and there was no relationship detected between the two parameters. However, many researchers have reported that a moderate to a low relationship was detected between increased BHBA levels and decreased milk yield, insufficient reproduction, the rate of abomasal displacement, metritis, and mastitis [23]. Although, after parturition, many cows have high NEFA levels, there is a poor correlation between NEFA and BHBA levels [24]. In addition, it was determined that the application of calcitriol (an active form of vitamin D) to dairy cows in the postpartum period caused an increase in BHBA level and reported results in parallel with the results of our study [18].

Vitamin D levels generally change between 40 and 100 ng/mL in Holstein cows in the different lactation periods [25]. In addition, they also reported that in the cows that received 30.000–50.000 IU vitamin D daily, vitamin D levels were generally found to be approximately 70 ng/mL. According to the results obtained in our study, although the vitamin D levels of the cows were generally detected in the normal range, the vitamin D levels of the cows were determined to be low when compared to the other studies' data [25, 26]. Vitamin D levels were determined significantly higher on the 7th, 14th, and 28th days in the EG than in the CG ($p < 0.05$). However, a significant difference ($p < 0.05$) was determined between the 1st and 3rd days and the 14th and 28th days in the EG, and vitamin D levels increased from the 7th to the 28th days. Thus, after intramuscular administration of 5,000,000 IU vitamin D, the increase in serum levels continued at the highest level in the blood for at least 1 month starting 3 days after administration. It can be suggested that the time interval between two doses of vitamin D should not be less than one month.

In this study, it has been shown that there was a strong and positive correlation between vitamin D and vitamin A and total cholesterol. This condition can be explained with the effects of vitamin D on the liver and NEFA as in the relationship between calcium and these parameters [27,28]. Although no statistically significant difference was detected between the two groups, the EG's mean LFI

value (–2.76 ± 0.82) is 1.44-fold higher than the mean value of the CG (–3.98 ± 1.37) and this result is compatible with the formula [4]. There are significant positive correlations detected between total cholesterol, which is one of the parameters used calculating LFI, and calcium, vitamin D ($p < 0.05$), and a significantly strong negative correlation between NEFA ($p < 0.01$). Furthermore, there was a significant negative correlation detected between bilirubin, which is another parameter used for calculating LFI and NEFA ($p < 0.05$). Thus, in addition to vitamin D's positive effects on NEFA, it can be concluded that it improves liver functions by means of reducing insulin resistance via direct and indirect mechanisms [27-29]. In another study, the authors separated cows into two groups as high LFI group (+2.5 ± 1.0) and low LFI group (–4.9 ± 1.3) [30]. Although it was reported that the difference in LFI scores of the groups is more prominent than our results, no statistically significant difference was detected between the groups regarding milk production as in our study. Furthermore, it was reported that, as in our study, NEFA concentrations of the cows in the low LFI group were higher than the cows in the high LFI group [30]. In contrast to our study, they reported the mean BHBA level of the low LFI group was higher than the mean BHBA level of the high LFI group. While in the EG, haptoglobin levels started to increase on the 3rd day and started to decrease from the 7th day, finally, the 28th-day haptoglobin levels of both the groups were determined to be nearly the same, making it compatible with the previous reports [31]. The significant and strong negative correlation ($p < 0.01$) between calcium and Hp and SAA can be explained via the hypothesis that the level of inflammatory parameters decreases after functions of neutrophil increase parallel with the calcium level [3, 32]. A decrease was determined in phagocytosis and oxidative burst functions in the neutrophils of subclinical hypocalcemic cows and related with that they reported an increase in the proportion both metritis and mastitis [3]. Similarly, a decrease was determined in neutrophilic functions after induction of hypocalcemia in dairy cows, and they reported that there was negative correlation between the calcium levels not only neutrophil functions but also, compatible with our results, plasma nonesterified fatty acids [33].-

There was a negative correlation between haptoglobin and vitamin A ($p < 0.01$). This negative correlation can be explained via Hp being a positive kind of APP and vitamin A being a negative kind of APP [34]. Additionally, it can be suggested that the SAA level increase in the 1st week is compatible with the previous reports. On the other hand, when SAA levels were compared in both groups, a significant ($p < 0.05$) increase was observed in the EG only on the 3rd day. This condition may result from decreased neutrophil functions [3,5] as a result of SCH. In parallel with this, calcium was detected at the lowest level on the 3rd day in the EG. Significant negative correlations ($p < 0.01$) were detected between SAA, calcium, and vitamin A; it can be suggested that the reasons are the same reasons that have an effect on haptoglobin.

It was shown that NEFA levels of cows that have high haptoglobin levels and low LFI was higher than the cows that have low haptoglobin levels and high LFI [32]. In another study, it was observed that Hp, SAA, and NEFA levels were higher in cows with ketosis than the control cows [35]. Furthermore, it was reported that serum Hp, SAA, and NEFA levels were higher in cows with fatty liver disease than in the control group [36]. In our study, the results between NEFA, Hp, and SAA were compatible with previous studies.

The low number of cows included in the study, the lack of ionized calcium levels, which are important in the assessment of calcium metabolism and immune functions, are some deficiencies. On the other hand, taking blood samples from cows at 5 different times in the first 28 days of the lactation period and evaluating the liver functions of cows based on liver-specific indices calculated using liver-specific parameters can be considered strengths of this study. It can be suggested that this study is important in that it is the first study in which the effects of intramuscular vitamin D administration on liver functions were evaluated numerically and it investigated the relationship between vitamin D and NEFA in dairy cows at the beginning of the lactation period.

5. Conclusions

In conclusion, we observed that the administration of vitamin D 24 h postpartum can cause a transient increase in the incidence of SCH, but it can decrease SCH incidence for up to 14 days. However, further studies with vitamin D may be recommended to prevent subclinical hypocalcemia in the first 3 days. On the other hand, it was observed that vitamin D affects NEFA levels positively. In addition, we concluded that while vitamin D has no effect on the LAI in dairy cows in the postpartum period, it has a limited positive effect on LFI.

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