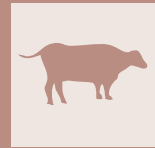


Investigation of the Relationship Between Vitamin D Levels, Fibrinogen, and Platelet-to-Lymphocyte Ratio (PLR) in Neonatal Calves with Diarrhea



TAHIR OZALP*¹, HASAN ERDGAN¹, SONGUL ERDOGAN¹, KEREM URAL¹

¹ Aydın Adnan Menderes University, Faculty of Veterinary Medicine, Department of Internal Medicine, 09100, Aydın, Turkey

SUMMARY

Neonatal calf diarrhoea (NCD) causes significant economic losses due to high mortality rates and growth retardation, with both infectious and non-infectious factors playing a role in its etiology. The infectious agents include coronavirus, rotavirus, *Cryptosporidium* spp., *Eimeria*, *Escherichia coli*, and *Salmonella enterica*, whereas the non-infectious factors comprise milk deficiency, vitamin deficiencies, and abrupt alterations in feeding practices. Biomarkers such as vitamin D, fibrinogen, and platelet-to-lymphocyte ratio (PLR) are important in inflammatory processes, with low vitamin D levels being associated with both acute and chronic disease states. This study aims to determine the relationship between vitamin D levels, fibrinogen, and PLR values, evaluating their potential diagnostic and prognostic utility in calves with neonatal diarrhoea, offering the possibility of identifying new, non-invasive tools for detecting non-infectious diseases. The study involved 80 diarrhoeic and 20 healthy Holstein calves, with clinical evaluations performed to assess dehydration status, vital parameters, and hematological profiles. In the infected group, calves were classified as mono-infected when at least one pathogen, such as *Cryptosporidium* spp., coronavirus, rotavirus, *E. coli*, or *Giardia* spp., was detected. The presence of multiple pathogens in the same animal was considered co-infection. Blood samples from both groups were analyzed for platelet-to-lymphocyte ratio, 25-hydroxyvitamin D₃, and fibrinogen levels. The study found lower vitamin D and higher fibrinogen levels in infected calves. Pathogen-specific differences were observed, with lower vitamin D and higher fibrinogen in *Cryptosporidium* spp. and *E. coli*-*Cryptosporidium* spp. co-infections, and rotavirus infection linked to thrombocytopenia and lower PLR. PLR showed the highest diagnostic accuracy in *Giardia* and *Cryptosporidium* spp. co-infections. This study highlighted the role of vitamin D deficiency, fibrinogen concentration, and the PLR index in modulating immune responses and inflammation in neonatal calf diarrhoea, suggesting that PLR could serve as a valuable biomarker for assessing inflammation severity under field conditions, with further researches needed to confirm its clinical applicability.

KEY WORDS

Calf; Diarrhea; Fibrinogen; Platelet to lymphocyte ratio; Vitamin D.

INTRODUCTION

Neonatal calf diarrhoea (NCD) causes serious economic losses in animal production due to problems such as high mortality rates and growth retardation in treated calves (1,2). Infectious and non-infectious factors such as insufficiency in milk replacers, vitamin deficiencies and sudden changes in the feeding system play a role in the aetiology of the disease (3). Infectious agents causing neonatal calf diarrhoea include viruses (e.g. rotavirus, bovine coronavirus, bovine viral diarrhoea virus [BVDV]), protozoa (e.g. *Cryptosporidium* spp. and *Eimeria* spp.) and bacteria (e.g. enterotoxigenic, enteropathogenic, enterohemorrhagic *Escherichia coli* and *Salmonella enterica*) (4-7). Studies in human medicine suggest a potential association be-

tween vitamin D and acute diarrhoea (8). The active metabolite of vitamin D, 1,25-dihydroxyvitamin D (calcitriol), plays an important role in modulating immune system responses. In particular, calcitriol blocks B-cell proliferation, suppresses cell differentiation and decreases immunoglobulin secretion (9). It also reduces T-cell proliferation and inhibits dendritic cell differentiation and maturation (10). Serum 25-(OH)D levels were found to be decreased in patients with systemic inflammatory response syndrome (SIRS), indicating that vitamin D plays a role in inflammatory processes (11,12). In addition, it was reported that 25-(OH)D levels decreased in calves with diarrhoea while fibrinogen levels increased, suggesting that vitamin D can be considered as a negative acute phase biomarker and that vitamin D is an important biomarker in both acute and chronic disease states (13,14).

Fibrinogen, which plays an active role in the formation of fibrin, the key protein in blood clotting and coagulation, is a glycoprotein biomarker used in the determination of inflammatory processes (15,16). Fibrinogen is synthesised by hepatocyte

*Corresponding Author:
Tahir Ozalp (tozalp@adu.edu.tr)

cells of the liver as a component of the acute phase response in inflammatory processes (16). It is reported that fibrinogen levels are elevated in the first 4 days (usually on the 3rd or 4th day), which is the early period of inflammatory processes, and this shows the diagnostic value of fibrinogen in the inflammatory process (15). Platelet-to-lymphocyte ratio (PLR) is a parameter calculated with the help of complete blood count (CBC) tests, and when compared with white blood cell (WBC), it is a more reliable biomarker in determining inflammatory processes and PLR levels have been found to be high in patients with chronic inflammatory diseases such as autoimmune diseases, diabetes status, rheumatic diseases and cancers (17-19). In addition, high PLR values have been reported to be closely associated with high mortality in progressive cancer cases, acute pulmonary embolism and patients with gynecological tumours (18,19). This study aims to determine the relationship between vitamin D levels, fibrinogen and PLR values. By examining these biomarkers as a group, it is aimed to evaluate the potential diagnostic and prognostic utility of these biomarkers in calves with neonatal diarrhoea. This view will offer the possibility of identifying new, non-invasive tools for the detection of non-infectious diseases.

MATERIALS AND METHODS

Study Design and Groups

Our study was conducted between 2019 and 2024 in the Internal Medicine Clinic of Aydın Adnan Menderes University Faculty of Veterinary Medicine Animal Hospital. A total of 100 Holstein calves, including 80 sick calves aged 1-28 days (infected group) and 20 healthy calves aged 1-28 days (control group), which were brought to the clinic and found to have acute diarrhoea in clinical examinations, were included in the study. It was evaluated in the infected group as mono-infected calves at least one pathogen must be found including *Cryptosporidium sp.*, coronavirus, rotavirus, *E. coli*, *Giardia sp.*, and more than one pathogen was associated with co-infection.

Blood Sampling and Laboratory Analysis

Blood samples were collected aseptically from the jugular vein into EDTA, heparin, and sodium citrate tubes (Vacutainer, Austria), with 4 mL collected per animal. The clinical examination of the infected animals included measurements of body temperature, dehydration levels (%), heart rate, and respiratory rate. Dehydration degree was assessed using a millimeter ruler to measure the distance of the eye from the orbital fossa and by evaluating skin elasticity. Complete blood counts were performed using an automated

hematology analyzer (Abacus Junior Vet, Hungary), with blood sample of anticoagulant EDTA for determining PLR following absolute platelet/lymphocyte ratio. Heparinized and sodium citrate plasma samples were separated via centrifugation at 3000 rpm for 5 minutes (Hettich, Germany). and were kept at -20°C until the 25-hydroxyvitamin D₃ (25-OH-D₃) and fibrinogen analysis. 25-OH-D₃ levels were analyzed using the fluorescent immunochromatographic method with a fluorescent immunoassay device (Savant Beijing Savant Biotechnology, China) from heparinized plasma with commercially available test. Fibrinogen levels were determined from sodium citrate plasma using a semi-automatic coagulometer (Semi-Automatic Blood Coagulation Analyzer C2000-4) by procedure of manufacture.

Statistical Analysis

Statistical analyses were performed using SPSS 29.0 software (IBM Corp., USA). The distribution of variables was evaluated by Shapiro-Wilk test. Mann-Whitney U test was used for intergroup comparisons for non-normally distributed data. Kruskal-Wallis test was used to compare 25-OH vitamin D levels, fibrinogen, PLR and other hematologic parameters in infected subgroups according to pathologic agents; in cases where significant differences were found, post-hoc pairwise comparisons were made with Dunn-Bonferroni correction. The relationships between variables were evaluated by Spearman correlation analysis. Logistic regression analysis was performed to evaluate the discriminative power of PLR and other hematologic/biochemical parameters between healthy and patient groups. Regression coefficients obtained from these analyses were interpreted. In addition, Receiver Operating Characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of PLR. The area under the curve (AUC), sensitivity, specificity and diagnostic accuracy of the PLR were calculated for each pathogen group. Optimal cut-off values were determined based on the Youden index. Statistical significance level was accepted as $p < 0.05$ in all tests. Visualization of the data was performed using ggplot plots packages.

RESULTS

Significant differences in various biochemical and hematological parameters were identified between the infected and healthy groups (Table 1). Vitamin D levels were significantly lower in the infected group (37.86 ± 15.94 ng/mL) compared to the healthy group (65.86 ± 12.57 ng/mL; $p < 0.05$). Similarly, fibrinogen levels were markedly higher in the infected group (292.40 ± 83.80 mg/dL) than in the healthy group (126.37 ± 37.23 mg/dL; $p < 0.05$).

Table 1 - Hematological parameters of healthy and infected calves (overall infection, irrespective of etiological agent).

Group	Mean $\pm$ SD					
	Vit D	Fibrinogen	WBC	LYM	PLT	PLR
Infected calves (n=80)	37.86 $\pm$ 15.94 ^a	292.40 $\pm$ 83.80 ^b	18.74 $\pm$ 12.78 ^b	4.24 $\pm$ 1.90	539.76 $\pm$ 361.79 ^a	144.56 $\pm$ 118.72
Healthy calves (n=20)	65.86 $\pm$ 12.57 ^b	126.37 $\pm$ 37.23 ^a	10.01 $\pm$ 3.40 ^a	4.74 $\pm$ 1.10	884.15 $\pm$ 1428.45 ^b	179.49 $\pm$ 262.90

^{a,b}: Values denoted by different letters within the same column are statistically significantly (0,001)., ns: No significant difference; no superscript letters are shown.

White blood cell (WBC) counts were significantly elevated in the infected group ($18.74 \pm 12.78 \times 10^3/\mu\text{L}$) compared to the healthy group ($10.01 \pm 3.40 \times 10^3/\mu\text{L}$; $p < 0.05$). However, no significant difference was observed in lymphocyte (LYM) counts between the infected ($4.24 \pm 1.90 \times 10^3/\mu\text{L}$) and healthy groups ($4.74 \pm 1.10 \times 10^3/\mu\text{L}$; $p > 0.05$). Platelet (PLT) counts in the infected group ($539.76 \pm 361.79 \times 10^3/\mu\text{L}$) were significantly lower compared to the healthy group ($884.15 \pm 1428.45 \times 10^3/\mu\text{L}$; $p < 0.05$). The PLR did not show a significant difference between the infected (144.56 ± 118.72) and healthy groups (179.49 ± 262.90 ; $p > 0.05$).

In this study, also these parameters were evaluated between enteropathogen(s) and significant differences were identified associated with agents (Table 2). 25-OH-D3 levels in the mono-infected calves with *Cryptosporidium* sp. (36.89 ± 12.81 ng/mL), rotavirus (34.48 ± 18.48 ng/mL), coronavirus (36.40 ± 22.57 ng/mL) were significantly lower compared to the healthy group. Similarly, significant reductions were observed in, co-infected calves with *E. coli-Cryptosporidium* sp. (37.42 ± 13.46 ng/mL) compared to the healthy ones ($p < 0.05$).

Fibrinogen levels were only significantly higher in the mono-infected calves with *Cryptosporidium* sp. (258.60 ± 95.60 mg/dL) and *E. coli-Cryptosporidium* co-infection (334.69 ± 44.29 mg/dL) compared to the healthy group ($p < 0.05$). A significant increase in WBC was observed in the mono-infected calves with *Cryptosporidium* sp. ($12.44 \pm 6.84 \times 10^3/\mu\text{L}$) and rotavirus ($23.01 \pm 14.29 \times 10^3/\mu\text{L}$) compared to the healthy group ($p < 0.05$), however only LYM was found to significant increase in the co-infected calves with *E. coli-Cryptosporidium* sp. (Table 2).

Compared to the healthy group, in the calves with rotavirus significantly thrombocytopenia ($p < 0.05$) was determined. Regarding platelet-to-lymphocyte ratio (PLR), significantly low-

er values were observed in several infected groups compared to the healthy calves (179.49 ± 262.90), including those infected with coronavirus (36.40 ± 22.57), *Cryptosporidium* sp. (36.89 ± 12.81), *Cryptosporidium-rotavirus* co-infection (34.04 ± 9.17), *E. coli-Cryptosporidium* sp. (48.49 ± 31.47), and *Cryptosporidium-rotavirus-coronavirus* co-infection (38.28 ± 8.99) ($p < 0.05$). These findings reveal significant differences in biochemical and hematological parameters associated with enteropathogens in the neonatal calf diarrhea (Table 1, Table 2).

The connection between PLR and vitamin D was weakly positive for the infected group ($r = 0.132$, $p > 0.05$) and weakly negative in the healthy group ($r = -0.259$, $p > 0.05$). These results show that there is not a statistically significant connection between the two factors tested. As a result of the analysis performed to determine the relationship between fibrinogen and PLR, no statistically significant correlation was detected between pathogen-specific infection, while a positive correlation was detected between these two parameters in calves infected with *Cryptosporidium* sp., rotavirus and corona virus ($r = 0.980$, $p > 0.05$) (Figure 1).

The relationship between hematological biomarkers was evaluated using logistic regression analysis. Fibrinogen biomarker showed a positive regression coefficient (-0.8), suggesting a negative relationship between LYM levels and fibrinogen levels. PLR showed a low regression coefficient and did not show a statistically significant relationship with LYM levels (Figure 2). Table 3 evaluates the diagnostic performance of PLR in neonatal diarrheal calves infected with different pathogenic agents. The diagnostic accuracy of PLR for *Giardia* sp. + *Cryptosporidium* sp. co-infected calves was found to be quite reliable (AUC = 0.92, Se: 100% and Sp: 92%). In parallel with this, *Giardia* infection showed a good diagnostic accuracy with an

Table 2 - Hematological parameters of calves infected with specific enteropathogens compared with healthy controls.

Enteropathogens	Mean $\pm$ SD					
	Vit D	Fibrinogen	WBC	LYM	PLT	PLR
Coronavirus (n=6)	36.40 $\pm$ 22.57 ^b	339.85 $\pm$ 47.88	23.40 $\pm$ 16.23	3.89 $\pm$ 1.38	104.47 $\pm$ 94.59	36.40 $\pm$ 22.57 ^b
<i>Cryptosporidium</i> sp. (n=17)	36.89 $\pm$ 12.81 ^b	258.60 $\pm$ 95.60 ^b	12.44 $\pm$ 6.84 ^b	4.33 $\pm$ 2.08	201.55 $\pm$ 134.50	36.89 $\pm$ 12.81 ^b
<i>Cryptosporidium</i> sp. +Rotavirus (n=17)	34.04 $\pm$ 9.17	274.32 $\pm$ 75.99	27.15 $\pm$ 15.23	3.70 $\pm$ 1.52	104.86 $\pm$ 113.88	34.04 $\pm$ 9.17 ^b
<i>Cryptosporidium</i> sp. + <i>E. coli</i> (n=12)	48.49 $\pm$ 31.47 ^b	338.02 $\pm$ 52.22 ^b	10.24 $\pm$ 1.60	4.37 $\pm$ 1.41 ^b	87.35 $\pm$ 19.47	48.49 $\pm$ 31.47 ^b
<i>Cryptosporidium</i> sp. +Rotavirus+Coronavirus (n=4)	38.28 $\pm$ 8.99	360.83 $\pm$ 81.71	8.58 $\pm$ 3.32	5.00 $\pm$ 1.55	154.74 $\pm$ 52.82	38.28 $\pm$ 8.99 ^b
<i>E. coli</i> (n=7)	51.70 $\pm$ 16.23	246.09 $\pm$ 126.88	13.90 $\pm$ 5.24	4.02 $\pm$ 1.44	205.14 $\pm$ 149.51	51.70 $\pm$ 16.23
<i>Giardia</i> spp. (n=5)	42.91 $\pm$ 26.09	296.72 $\pm$ 115.48	15.79 $\pm$ 11.87	4.67 $\pm$ 1.86	188.19 $\pm$ 101.72	42.91 $\pm$ 26.09
<i>Giardia</i> spp. + <i>Cryptosporidium</i> sp. (n=1)	27.17	368.60	7.31	2.52	334.13	27.17
Rotavirus (n=11)	34.48 $\pm$ 18.48 ^b	306.76 $\pm$ 62.28	23.01 $\pm$ 14.29 ^b	3.87 $\pm$ 2.27	82.50 $\pm$ 75.40 ^b	34.48 $\pm$ 18.48
Healthy (n=20)	65.86 $\pm$ 12.57 ^a	126.37 $\pm$ 37.23 ^a	10.01 $\pm$ 3.40 ^a	4.74 $\pm$ 1.10 ^a	884.15 $\pm$ 1428.45 ^a	179.49 $\pm$ 262.90 ^a

^{a,b}: Superscript letters (a, b) indicate statistically significant differences compared with the healthy control group within the same column ($p < 0.001$). Groups without superscripts are not significantly significant from the healthy control.

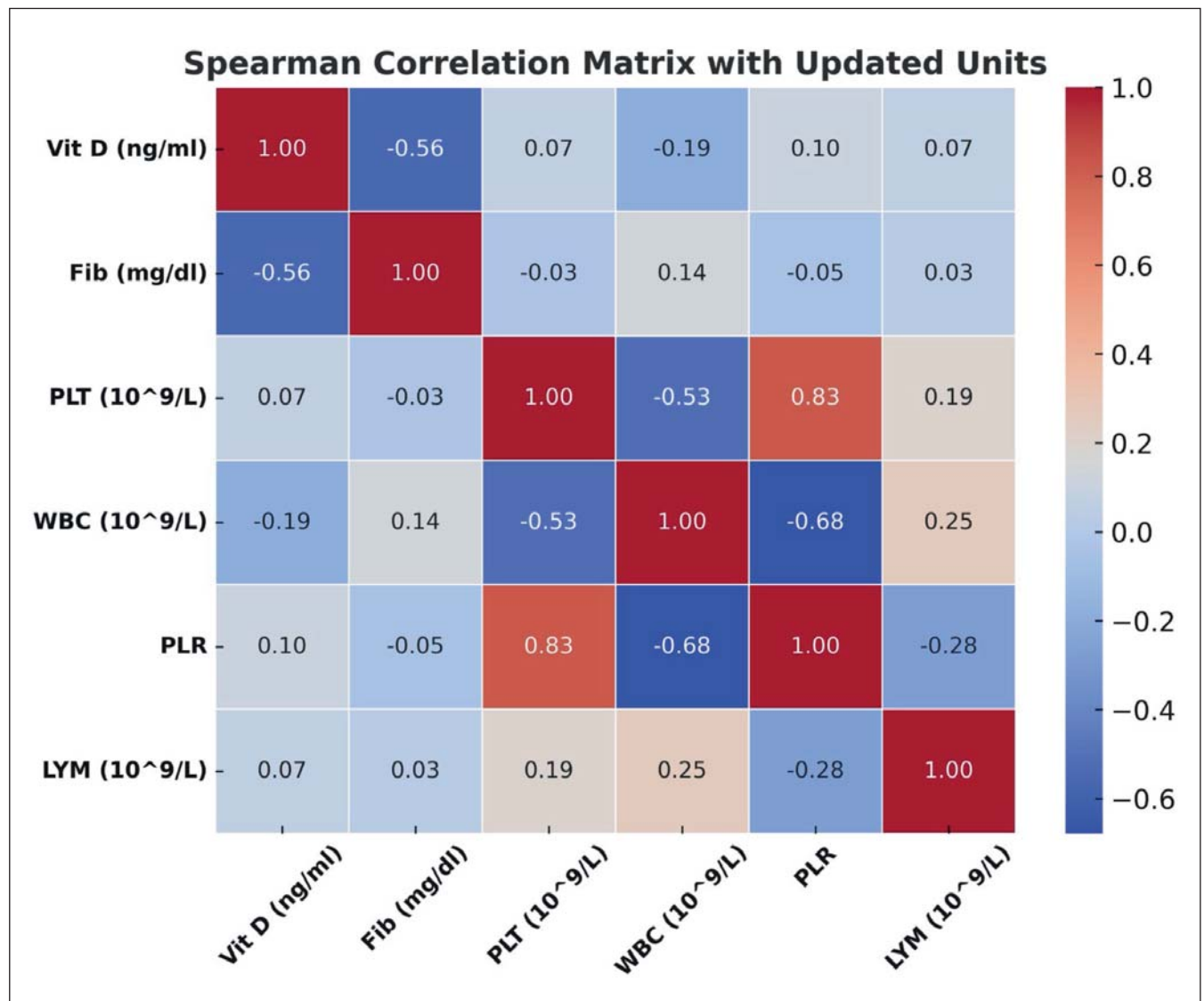


Figure 1 -Hematologic parameters and correlation.

AUC value of 0.75. In contrast, the diagnostic accuracy of PLR for *Cryptosporidium* (AUC = 0.66) and *E. coli* (AUC = 0.65) was found to be moderate, suggesting that this parameter would be more useful when used in conjunction with other diagnostic

tools. However, the diagnostic value of PLR was found to be weak for rotavirus (AUC = 0.31) and coronavirus (AUC = 0.41) infections, indicating that PLR is not a sufficient marker for the detection of these pathogens (Table 3, Figure 2).

Table 3 - Pathogen-specific PLR cut-offs and diagnostic efficacy in calf diarrhea.

Enteropathogens	AUC	Optimal Cut-off	Sensitivity	Specificity
<i>Cryptosporidium</i> sp.	0.66	66.95	1	0.28
Rotavirus	0.31	1284.4	0	1
<i>E.coli</i> + <i>Cryptosporidium</i> sp.	0.43	20	1	0.08
Coronavirus	0.41	225.26	0.29	0.84
<i>E. coli</i>	0.65	101.12	1	0.4
<i>Giardia</i> sp.	0.75	185.61	1	0.75
<i>Cryptosporidium</i> sp. + Rotavirus	0.55	73.59	1	0.32
<i>Giardia</i> + <i>Cryptosporidium</i> sp.	0.92	334.13	1	0.92
<i>Cryptosporidium</i> + <i>Rotavirus</i> + <i>Coronavirus</i>	0.6	113.67	1	0.46
Healthly	0.52	75.83	0.9	0.37

DISCUSSION

Neonatal calf diarrhea is an important health problem in cattle breeding and its high mortality causes serious economic losses and can also lead to long-term negative effects on treatment costs and growth performance, survival and general health status of treated calves. *Salmonella spp.*, Rotavirus, Coronavirus, *Cryptosporidium spp.*, and *E. coli* K99 (F5) are examples of pathogens that produce malabsorptive diarrhea and contribute to these losses (20). By stimulating the immune system response, infectious agents activate acute phase responses and systemic inflammation (21).

In our study, vitamin D levels in calves in the diseased group (37.86 ± 15.94 ng/mL) were found to be statistically significantly lower than in calves in the healthy group (65.86 ± 12.57 ng/mL) ($p < 0.05$). It has been reported that vitamin D plays a vital role both in the protection of the intestinal epithelial barrier and in the strengthening of its integrity (22). Vitamin D receptors (VDR) reduce intestinal epithelial surface permeability by regulating tight junction proteins in intestinal epithelial cells. Vitamin D deficiency is reported to be associated with epithelial barrier dysfunction, increased susceptibility to intestinal pathogens, and exacerbated inflammation (20). In our study,

Fibrinogen was significantly higher in stratified calves (292.40 ± 83.80 mg/dL) than in healthy calves (126.37 ± 37.23 mg/dL) ($p < 0.05$). Fibrinogen, which plays a role in acute phase responses, is a glycoprotein that plays a critical role in hemostasis and tissue maintenance components (23). Fibrinogen, produced together with the inflammatory process, can delay the elimination of an important role for the immune response when excessively increased. The negative relationship between vitamin D and fibrinogen is similar to the role of vitamin D/VDR signaling in storing NF- κ B fragments and reducing proinflammatory cytokine production (24). When the hematological effect in neonatal calves with diarrhea was evaluated, increases in WBC levels, decreases in PLT levels were observed, while significant changes were observed in other hematological effects. When we evaluated the PLR index, which is an important parameter in evaluating the degree of inflammation and prognosis (25), in our study, there were no significant differences between the patient group and the control group. The PLR index in human medicine has been investigated in detail in the diagnosis and prognosis of sepsis, acute gastroenteritis and other inflammatory disorders (25-27). It is reported that the PLR index can be used in the separation of viral and fragments as etiological agents, especially in pediatric acute gastroenteritis

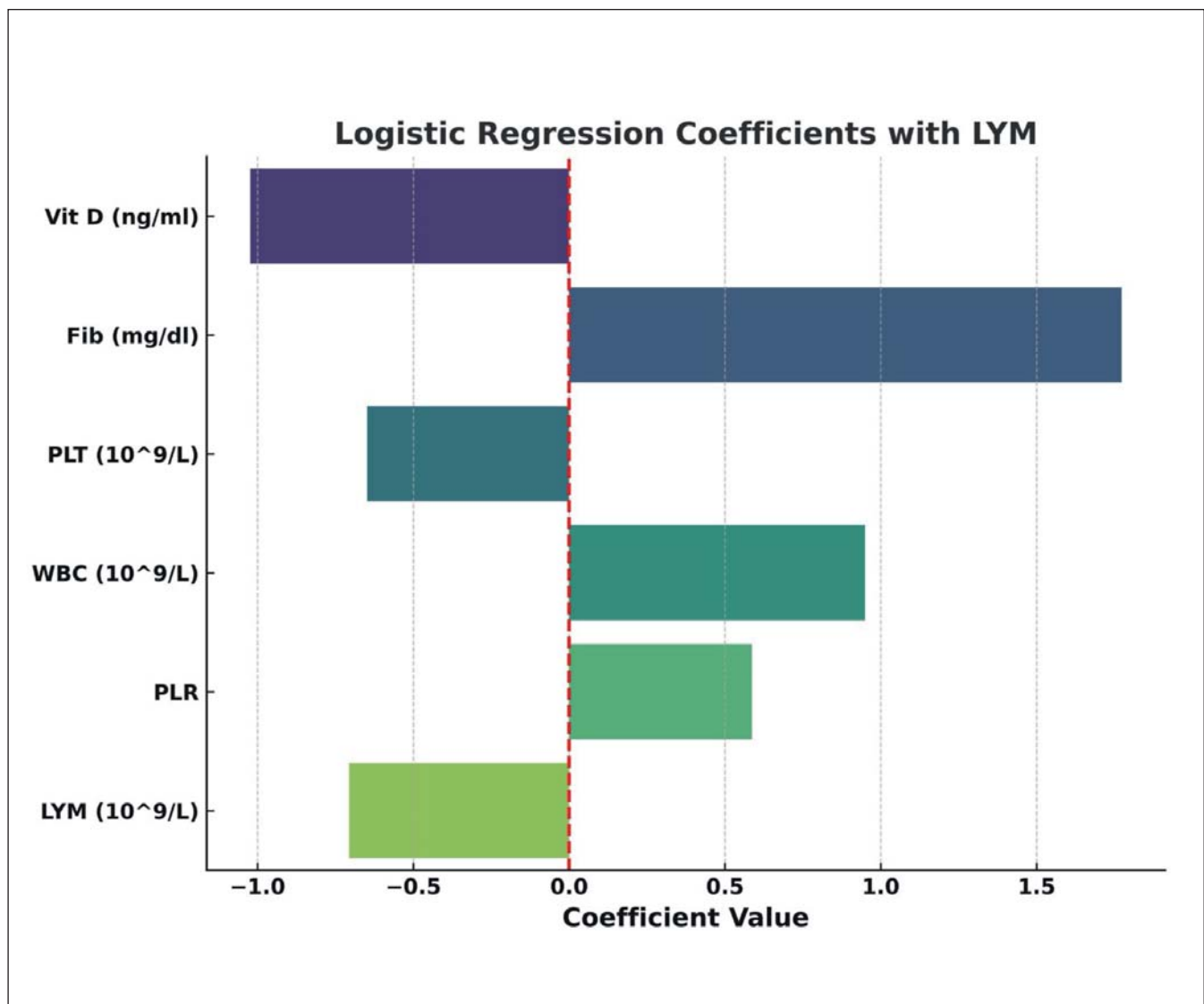


Figure 2 - Logistic regression analyses of hematological parameters.

(24). Despite this, there are limited studies in veterinary medicine that comprehensively investigate the PLR index in terms of diagnosis and prognosis (28-30).

In the light of the findings obtained in our study, the AUC values for the PLR index according to the types of pathogens were determined as follows: *Cryptosporidium spp.* (0.66), rotavirus (0.31), coronavirus (0.41), *E. coli* (0.65) and *E. coli* + *Cryptosporidium spp.* (0.35). These values were considered to have diagnostically important potential and the AUC value for calves in the healthy group was 0.52. The fact that changes were observed in sensitivity and specificity values showed that the AUC value may have some additional benefits. demonstrated the different usefulness of the parameter among pathogens. To clarify this issue, the sensitivity for *Cryptosporidium spp.* was 1.00, while the specificity was 0.28. The cutoff value for PLR index varied between 66.95 for *Cryptosporidium spp.* pathogens and 1284.40 for Rota virus pathogen, and the variation in values according to the agent emphasises the importance of diagnostic variability. When we look at the sensitivity and specificity results, high sensitivity and low specificity were observed for *Cryptosporidium spp.* pathogen, while low sensitivity and high specificity were observed for Rota virus, which further emphasises the importance of evaluating PLR according to pathogens.

The cutoff value for PLR varied between pathogen species, with significant differences between *Cryptosporidium spp.* (66.95) and Rota virus (1284.40). In the light of these findings, PLR index may be a diagnostically supportive marker, but it should be supported by pathogen-specific tests and interpretations for clinical accuracy.

The positive correlation between PLR and fibrinogen in *Cryptosporidium spp.* and *E. coli* pathogens reveals the role of acute phase proteins in inflammation. Although these relationships are not statistically significant, they are in line with the existing interpretations regarding the contribution of fibrinogen to systemic inflammatory responses.

The PLR index value is easy to determine and is cost-effective, making it an important biomarker in assessing the severity of inflammation, especially when used together with other biomarkers such as fibrinogen and WBC. However, the variability obtained in the study emphasizes the need for further research to confirm its diagnostic potential in routine applications and NCD cases. When the regression analysis findings were evaluated, they revealed that levels of fibrinogen showed a statistically significant positive influence on LYM levels, with a regression coefficient of around 1.5. This example depicts how higher fibrinogen levels can result in a rise in LYM numbers. PLT and WBC values had favorable correlations with LYM levels (coefficients of around 0.8 and 0.6, respectively). In contrast, a negative regression coefficient (approximately -0.8) was detected between vitamin D levels and LYM levels. This situation is explained by the fact that low levels of Vitamin D may affect the role of regulating immune system function and may cause an increase in the number of lymphocyte cells. PLR index levels did not show a statistically significant relationship with lymphocyte-related components, which suggests that the effect of PLR may be limited or may be affected by other biochemical or hematological parameters.

Platelets mainly affect the immune system by regulating the release of proinflammatory and anti-inflammatory cytokines and cooperating with neutrophils and lymphocytes (Kriplani et al., 2022). When all these results are evaluated, it is revealed that

PLR index can be a rapid, cost-effective and applicable biomarker in the evaluation of inflammation severity in calves with diarrhoea in the neonatal period, shorten the diagnostic process and facilitate clinical decision-making.

CONCLUSION

This study investigated in detail the effect of Vitamin D deficiency, Fibrinogen concentration, PLR index on the immune system and inflammatory status in neonatal calf diarrhoea. When the findings were evaluated, it was concluded that serum vitamin D level can modulate the immune system when it is at adequate level and the potential diagnostic effect of PLR in field conditions where adequate analyses cannot be performed. The results show that the PLR index is a supportive biomarker in determining the severity of inflammation and will make an important contribution in veterinary medicine clinical applications. In particular, when the relationships between vitamin D levels, fibrinogen levels and PLR index are evaluated, it is emphasised that these parameters are interdependent in immune regulation in cases of inflammation. Further studies with larger samples are needed to strengthen the clinical applicability of PLR and other haematological biomarkers in the regulation of inflammatory responses in calf diarrhoea.

Conflict of interest

There is no conflict of interest between the authors of this article.

Ethical Approval Statement

This study was approved by the Aydın Adnan Menderes University Local Ethics Committee for Animal Experiments (ADÜ-HADYEK), under approval number: 64583101/2025/047.

References

1. De Graaf, D. C., Vanopdenbosch, E., Ortega-Mora, L. M., Abbassi, H., and Peeters, J. E. 1999. A review of the importance of cryptosporidiosis in farm animals. *Int. J. Parasitol.*, 29(8):1269-1287.
2. Häslar, B., Howe, K. S., Presi, P., and Stärk, K. D. C. 2012. An economic model to evaluate the mitigation programme for bovine viral diarrhoea in Switzerland. *Prev. Vet. Med.*, 106(2):162-173.
3. Cho, Y. I., Han, J. I., Wang, C., Cooper, V., Schwartz, K., Engelken, T., and Yoon, K. J. 2013. Case-control study of microbiological etiology associated with calf diarrhea. *Vet. Microbiol.*, 166(3-4):375-385.
4. Cho, Y. I., and Yoon, K. J. 2014. An overview of calf diarrhea - infectious etiology, diagnosis, and intervention. *J. Vet. Sci.*, 15:1-17.
5. De La Fuente, R., Garcia, A., Ruiz-Santa-Quiteria, J. A., Luzón, M., Cid, D., García, S., et al. 1998. Proportional morbidity rates of enteropathogens among diarrheic dairy calves in central Spain. *Prev. Vet. Med.*, 36:145-152.
6. Gomez, D. E., and Weese, J. S. 2017. Viral enteritis in calves. *Can. Vet. J.*, 58:1267-1274.
7. Uzal, F., Plattner, B., and Hostetter, J. 2016. Alimentary system. In: Maxie, G. (ed.) *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*. St. Louis, Missouri, USA: Elsevier, pp. 1-257.
8. Lazarus, G., Putra, I. G. N. S., Junaidi, M. C., Oswari, J. S., and Oswari, H. 2024. The relationship of vitamin D deficiency and childhood diarrhea: a systematic review and meta-analysis. *BMC Pediatr.*, 24(1):125.
9. Chen, S., Sims, G. P., Chen, X. X., et al. 2007. Modulatory effects of 1,25-dihydroxy vitamin D3 on human B cell differentiation. *J. Immunol.*, 179(3):1634-1647.
10. Széles, L., Keresztes, G., Töröcsik, D., et al. 2009. 1,25-dihydroxy vitamin D3 is an autonomous regulator of the transcriptional changes leading to a tolerogenic dendritic cell phenotype. *J. Immunol.*, 182(4):2074-2083.
11. Louw, J. A., Werbeck, A., Louw, M. E., et al. 1992. Blood vitamin concentrations during the acute-phase response. *Crit. Care Med.*, 20:934-941.

12. Reid, D., Toole, B. J., Knox, S., et al. 2011. The relation between acute changes in the systemic inflammatory response and plasma 25-hydroxyvitamin D concentrations after elective knee arthroplasty. *Am. J. Clin. Nutr.*, 93:1006-11.
13. Erdo an, H., and Özalp, T. 2019. Neonatal ishali buza ılda 25(OH)D3 konsantrasyonlarının araştırılması. *Kocatepe Vet. J.*, 12:268-276.
14. Waldron, J. L., Ashby, H. L., Cornes, M. P., Bechervaise, J., Razavi, C., Thomas, O. L., et al. 2013. Vitamin D: a negative acute phase reactant. *J. Clin. Pathol.*, 66:620-622.
15. Conner, J. G., Eckersall, P. D., Wiseman, A., Aitchison, T. C., and Douglas, T. A. 1988. Bovine acute phase response following turpentine injection. *Res. Vet. Sci.*, 44:82-88.
16. Eckersall, P. D., and Conner, J. G. 1988. Bovine and canine acute phase proteins. *Vet. Res. Commun.*, 12:169-178.
17. Gasparyan, A. Y., Ayvazyan, L., Mukanova, U., Yessirkepov, M., and Kitas, G. D. 2019. The platelet-to-lymphocyte ratio as an inflammatory marker in rheumatic diseases. *Ann. Lab. Med.*, 39:345-357.
18. Li, B., Zhou, P., Liu, Y., et al. 2018. Platelet-to-lymphocyte ratio in advanced cancer: review and meta-analysis. *Clin. Chim. Acta*, 483:48-56.
19. Jiang, S., Liu, J., Chen, X., et al. 2019. Platelet-lymphocyte ratio as a potential prognostic factor in gynecologic cancers: a meta-analysis. *Arch. Gynecol. Obstet.*, 300:829-839.
20. Foster, D. M., and Smith, G. W. 2009. Pathophysiology of diarrhea in calves. *Vet. Clin. North Am. Food Anim. Pract.*, 25:13-36.
21. Pfeffer, A., Rogers, K. M., O'Keeffe, L., and Osborn, P. J. 1993. Acute phase protein response, food intake, liveweight change and lesions following intrathoracic injection of yeast in sheep. *Res. Vet. Sci.*, 55:360-366.
22. Albert Bayo, M., Abril-Gil, M., Ganda Mall, J. P., Serra Ruiz, X., Henao Paez, A., Exposito Fernandez, E., et al. 2021. Present and future therapeutic approaches to barrier dysfunction.
23. Murata, H., Shimada, N., and Yoshioka, M. 2004. Current research on acute phase proteins in veterinary diagnosis: an overview. *Vet. J.*, 168:28-40.
24. Okuyan, O., Elgormus, Y., Sayili, U., Dumur, S., Isik, O. E., and Uzun, H. 2023. The effect of virus-specific vaccination on laboratory infection markers of children with acute rotavirus-associated acute gastroenteritis. *Vaccines*, 11:580.
25. Wang, R. H., Wen, W. X., Jiang, Z. P., Du, Z. P., Ma, Z. H., Lu, A. L., et al. 2023. The clinical value of neutrophil-to-lymphocyte ratio (NLR), systemic immune-inflammation index (SII), platelet-to-lymphocyte ratio (PLR) and systemic inflammation response index (SIRI) for predicting the occurrence and severity of pneumonia in patients with intracerebral hemorrhage. *Front. Immunol.*, 14:1115031.
26. Kriplani, A., Pandit, S., Chawla, A., de la Rosette, J. J., Laguna, P., Jayadeva Reddy, S., and Somani, B. K. 2022. Neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR) and lymphocyte-monocyte ratio (LMR) in predicting systemic inflammatory response syndrome (SIRS) and sepsis after percutaneous nephrolithotomy (PNL). *Urolithiasis*, 50:341-348.
27. Marazziti, D., Torrigiani, S., Carbone, M. G., Mucci, F., Flamini, W., Ivaldi, T., and Dell'Osso, L. 2022. Neutrophil/lymphocyte, platelet/lymphocyte, and monocyte/lymphocyte ratios in mood disorders. *Curr. Med. Chem.*, 29:5758-5781.
28. Manulboga, M., Tendar, A., Özalp, T., Erdogan, S., Ural, K., and Erdogan, H. 2024. Association of D-dimer-fibrinogen ratio, platelet-lymphocyte ratio, and vitamin D levels with pneumonia in calves: insights into inflammation and coagulation mechanisms. *Egypt. J. Vet. Sci.*, 55:49-57.
29. Aydın, Ö., and Yıldırım, B. A. 2024. Determination of systemic inflammation response index (SIRI), systemic inflammatory index (SII), HMGB1, Mx1 and TNF levels in neonatal calf diarrhea with systemic inflammatory response syndrome. *Vet. Immunol. Immunopathol.*, 275:110815.
30. Yanar, K. E., Eren, E., Aktaş, M. S., Ero lu, M. S., Kandemir, Ö., and Aydın, G. 2023. Prognostic potential of inflammatory markers, oxidative status, thrombocyte indices, and renal biochemical markers in neonatal calf diarrhoea-induced systemic inflammatory response syndrome. *Vet. Immunol. Immunopathol.*, 265:110680.