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Effects of zinc-reinforced clinoptilolite on hematological parameters, IgG levels, and enzyme histochemistry in fattening lambs

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Abstract: The study aimed to determine the effects of zinc-reinforced clinoptilolite supplementation to the diet on selected hematological parameters, serum immunoglobulin G (IgG) concentrations, and enzyme histochemical characteristics in fattening lambs. For this purpose, 40 healthy male lambs were randomly allocated into five equal groups: control (C; $n = 8$), natural clinoptilolite (NC; $n = 8$), zinc (ZN; $n = 8$), natural clinoptilolite plus zinc (NCZN; $n = 8$), and zinc-reinforced clinoptilolite (ZNRC; $n = 8$). All animals were housed in individual pens, and the supplementation period continued for 42 days. Blood samples were collected before the trial (day 0) and at the end of the trial (day 42) to evaluate hematological parameters, serum IgG concentrations, and enzyme activity. Lymphocyte and monocyte percentages increased only in the ZN group at day 42 compared with day 0 ($p < 0.05$). Mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and hemoglobin values decreased in all experimental groups at day 42 compared with day 0 ($p < 0.05$). Serum IgG concentrations increased at day 42 compared with day 0 in the ZN, NCZN, and ZNRC groups ($p < 0.05$). Furthermore, supplementation with zinc-reinforced clinoptilolite resulted in higher serum IgG concentrations in the ZNRC group compared with the other experimental groups ($p < 0.05$). Alpha-naphthyl acetate esterase-positive lymphocyte ratios were not affected by the different dietary treatments in any experimental group ($p > 0.05$). In conclusion, zinc-containing combinations (NCZN and ZNRC) appeared to exert more pronounced effects on immune-related parameters, particularly lymphocyte percentages and serum IgG concentrations. These findings suggest that alternative nutritional strategies may be developed by utilizing the ion-exchange capacity of clinoptilolite.

Key words: Clinoptilolite, lambs, zinc, hematological parameters, immunoglobulin, enzyme histochemistry

1. Introduction

Zeolites, including clinoptilolite, are crystalline inorganic microporous solids [1]. Volcanic regions, alkaline desert lakes, soils, and marine sediments constitute the primary natural sources of these aluminosilicates [2]. In recent years, zeolites have been widely used in various industrial fields, including biotechnology, medicine, chemistry, petrochemistry, and pharmaceuticals, due to their surface adsorption capacity, high ion-exchange capability, catalytic and molecular sieve properties, structural stability during hydration and dehydration, and low-density characteristics. They have also been characterized as sustainable, safe, and environmentally friendly materials [3,4]. As a result of these aforementioned properties, zeolite is widely utilized as a feed additive in animal nutrition worldwide. Its application as a dietary supplement has expanded due to its capacity to retain moisture and oil, inhibit mycotoxin production, and absorb ruminal ammonia [5,6]. Moreover, experimental animal studies have demonstrated that zeolites may contribute to detoxification processes, enhancement of immune function and nutritional status, separation of biomolecules and cell types, transport of nucleic acids and bioactive compounds, and scavenging of reactive oxygen species [7]. In summary, the reported biological effects of zeolites include antioxidant, immunostimulatory, growth-promoting, antidiarrheal, hemostatic, antibacterial, and antiviral activities [8,9]. Globally, major zeolite production occurs in Cuba, Japan, the United States, South Africa, Hungary, Bulgaria, and Italy. On the other hand, some Turkish regions such as İzmir, Bolu, Kütahya, Gördes, Balıkesir, Bigadiç, and Manisa also have considerable zeolite resources. Because zeolite is abundant and economically accessible, numerous studies have been conducted worldwide to expand its application as an animal feed additive [10]. The number of such studies has increased substantially, particularly with advances in nanotechnology [11,12].

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Zinc (Zn) is one of the most essential trace elements for maintaining the health of animals and humans. It is essential for optimal metabolic function due to its structural and catalytic roles in numerous enzymes and transcription factors. In particular, Zn plays a critical role in immune system activation. Furthermore, Zn is required for vitamin A metabolism, carbon dioxide transport, regulation of protein and carbohydrate metabolism, elimination of reactive oxygen species, and maintenance of erythrocyte membrane integrity. During periods of rapid growth, young ruminants—particularly lambs—require higher Zn intake to support protein accretion and enhance growth performance. Accordingly, Zn supplementation is provided to ruminants to meet mineral requirements and enhance immune and antioxidant defenses [13,14].

Blood parameters, including erythrocyte (RBC) and leukocyte (WBC) counts and their subtypes, platelet (PLT) count, hemoglobin (HGB), hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular volume (MCV), serve as a comprehensive screening panel for evaluating physiological status and detecting disease or infection in farm animals [15]. Previous studies have reported that zeolite supplementation does not adversely affect normal physiological and hematological homeostasis in small ruminants [16,17].

Immunoglobulin G (IgG), a major antibody isotype responsible for long-term humoral immunity, is the most abundant immunoglobulin in the blood and other tissues of many species, including sheep. Although IgG can be transferred from mother to fetus through the placenta in humans, placental transfer does not occur in ruminants and equids due to differences in placental structure. Consequently, newborn lambs are born hypogammaglobulinemic or agammaglobulinemic. Therefore, passive immunity in newborn lambs is provided primarily through colostrum intake [18]. Numerous studies have investigated the effects of adding clinoptilolite to ruminant diets on serum IgG concentrations [19,20].

Enzyme histochemical techniques have been widely applied in biomedical research and in the diagnosis of various disorders in recent years. These techniques provide critical information regarding enzyme identity, localization, and activity levels. Among these enzymes, alpha-naphthyl acetate esterase (ANAE), a lysosomal enzyme, is used in tissue sections and peripheral blood smears to differentiate T lymphocytes, B lymphocytes, and monocytes [21]. This enzyme is present in most mature and immature T lymphocytes. It is primarily localized within lysosomal membranes. Like other esterases, ANAE is involved in the degradation of phagocytosed material in macrophages and in the cytotoxic activity of activated T cells [22]. T and B lymphocyte ratios in the peripheral blood of healthy ruminants may vary considerably; however, these ratios can be significantly influenced by conditions such as proliferative diseases, immunonutritional deficiencies, and heavy metal toxicity. Little information is available regarding the effects of clinoptilolite applications on immune function in animals [22–24].

The objective of adsorbing Zn onto the clinoptilolite surface was to optimize Zn delivery, minimize Zn loss from the diet, and regulate Zn release through the ion-exchange capacity of clinoptilolite while facilitating the removal of undesirable components. Accordingly, this study aimed to evaluate the effects of zinc-reinforced clinoptilolite on selected hematological parameters, serum IgG concentrations, and ANAE-positive lymphocyte ratios in fattening lambs.

2. Materials and methods

2.1. Ethical approval

All experimental procedures were approved by the Balıkesir University Experimental Animal Ethics Committee (approval no: 2022/2–4).

2.2. Animals and feeding management

The study included a total of 40 healthy male lambs. The lambs were weighed at the beginning of the experiment (day 0) and randomly allocated into five equal groups based on mean live weight and age. They were approximately 60 days old, with a mean live weight of 23.02 ± 0.58 kg. The lambs were randomly assigned to five groups: (1) control (C; n = 8); (2) C + 25 g/kg natural clinoptilolite (NC; n = 8); (3) C + 0.375 g/kg zinc (ZN; n = 8); (4) C + 25 g/kg natural clinoptilolite + 0.375 g/kg zinc (NCZN; n = 8); and (5) C + 25.375 g/kg zinc-reinforced clinoptilolite (ZNRC; n = 8). The animals included in the trial were healthy, free of internal and external parasites, and had been vaccinated in line with the standard vaccination schedule for small ruminants.

The lambs were housed in the sheep breeding unit of Balıkesir University for 42 days. The study was conducted during the spring season in Balıkesir, where average daily temperatures ranged from 15 °C to 25 °C according to local meteorological data. No extreme weather conditions were observed during the trial. All lambs were housed in individual pens measuring 1.25 m × 1.80 m. Fresh drinking water was available ad libitum throughout the study. The primary feed used in the experiment was a pelleted lamb growth diet containing 16.32% crude protein. The basal diet was formulated according to the recommendations of the NRC [25] and subsequently pelleted. Experimental diets were offered ad libitum in two equal portions at 07:00 AM and 05:00 PM. All animals received roughage (alfalfa) at 15% of their total dry matter intake, which was offered in a separate feeder from the concentrate diet. Feed was provided and residual feed was collected daily.

There was no evidence of mold or spoiling in the feed. In the treatment groups, clinoptilolite and zinc oxide (ZnO) were included in the diets at levels of 25 g/kg (2.5%) and 0.375 g/kg, respectively. In the ZN group, 0.375 g of zinc was administered in liquid form. The inclusion levels of clinoptilolite were determined according to Katsoulos et al. [26], Abdelrahman et al. [27], and Sallam et al. [28], whereas zinc oxide levels were established based on NRC recommendations [25].

2.3. Preparation of zinc-reinforced clinoptilolite

Initially, the physicochemical properties of clinoptilolite obtained from the Balıkesir/Bigadiç region were investigated and characterized (Table 1). Following characterization, the natural mineral was incorporated into the feed formulations in accordance with relevant literature. Prior to these procedures, the clinoptilolite samples were crushed and sieved using a mill to obtain a particle size of less than 100 µm. The milled clinoptilolite was washed with purified water to remove surface and pore impurities. The cleaned particles were then dried in an oven at 105 °C for 24 h. Subsequently, zinc incorporation into the clinoptilolite was performed at the solid–liquid interface. In suspensions prepared at various solid–liquid ratios, Zn^{2+} ions were incorporated into the clinoptilolite matrix through diffusion, ion-exchange, and surface sorption mechanisms. The amount of Zn incorporated into the samples was determined by measuring Zn concentrations in both solid and liquid phases using inductively coupled plasma mass spectrometry. The specific formulations, compounds used, and details of the production process are proprietary and were withheld as confidential information [29].

2.4. Determination of hematological parameters

Blood samples were collected from the lambs at the beginning of the experiment (day 0) and at the end of the experiment (day 42). Samples were collected from the jugular vein using a 1.2 mm × 38 mm needle and transferred into heparinized tubes. Immediately after collection, the samples were transported to the laboratory under cold-chain conditions for analysis. The automated hematology analyzer (Mindray BC-5300; Mindray Bio-Medical Electronics Co Ltd., Shenzhen, China) was used to measure RBC and WBC counts and their subtypes, HGB, HCT, PLT count, MCV, MCH, and MCHC in plasma samples.

2.5. Determination of immunoglobulin G levels

Serum IgG concentrations were determined using the single radial immunodiffusion technique according to the manufacturer's instructions (Cat. no: E11–118; Bethyl Laboratories Inc., Montgomery, TX, USA).

2.6. Determination of ANAE-positive lymphocyte ratios

A total of two smears were prepared from each plasma sample. Air-dried smears were fixed for 3 min at 10 °C in a phosphate-buffered glutaraldehyde–acetone solution (pH 4.8). ANAE activity was demonstrated according to the method described by Dönmez et al. [30]. For each smear, 200 lymphocytes were counted, and cells exhibiting lymphocyte morphology with one to three large reddish-brown granules were identified as ANAE-positive lymphocytes (Figure) using a Nikon Eclipse 50i light microscope (Nikon Corporation, Tokyo, Japan).

2.7. Statistical analysis

The experiment was designed as a completely randomized design. The normality of the variables was assessed using the Shapiro–Wilk test. Analysis of variance (ANOVA) was performed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA). Group means were compared using Duncan's multiple range test, and within-group comparisons were performed using the paired-samples t test ($p < 0.05$).

3. Results

3.1. Hematological parameters

In the first analysis, no significant differences were observed among the experimental groups with respect to hematological parameters ($p > 0.05$). Lymphocyte (Lym) and monocyte (Mon) percentages increased only in

Table 1. Chemical composition of the natural clinoptilolite used in the study.

Ingredient	Percentage (%)
Silicon dioxide (SiO_2)	78.45
Aluminum oxide (Al_2O_3)	12.58
Iron (III) oxide (Fe_2O_3)	1.37
Magnesium oxide (MgO)	0.85
Calcium oxide (CaO)	3.12
Sodium oxide (Na_2O)	0.11
Potassium oxide (K_2O)	3.51
Phosphorus pentoxide (P_2O_5)	0.016

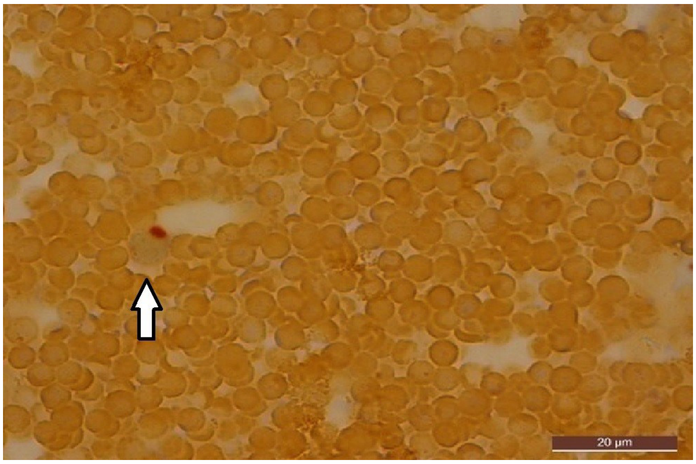

Figure. ANAE-positive lymphocyte in the control group.

the ZN group at day 42 compared with day 0 ($p < 0.05$). Additionally, Lym percentages were higher in the NCZN group than in the other experimental groups at day 42 ($p < 0.05$). RBC values decreased in the ZN and NCZN groups at day 42 compared with day 0 ($p < 0.05$). HCT values decreased only in the ZN group at day 42 compared with day 0 ($p < 0.05$). MCH, MCHC, and HGB values decreased in all experimental groups at day 42 compared with day 0 ($p < 0.05$), as shown in Table 2.

3.2. Serum IgG levels

At day 0, serum IgG concentrations were similar along the experimental groups ($p > 0.05$). Serum IgG concentrations increased at day 42 compared with day 0 in the ZN, NCZN, and ZNRC groups ($p < 0.05$). In the C and NC groups, serum IgG concentrations did not change significantly between day 0 and day 42 ($p > 0.05$). At day 42, as shown in Table 3, supplementation with zinc-reinforced clinoptilolite resulted in higher serum IgG concentrations in the ZNRC group compared with the other experimental groups ($p < 0.05$).

3.3. ANAE-positive lymphocyte ratios

ANAE-positive lymphocyte ratios were not affected by the different dietary treatments in any experimental group ($p > 0.05$), as shown in Table 4.

4. Discussion

A hemogram refers to a complete blood count that evaluates the number and characteristics of blood cells in animals. These parameters provide important information regarding the physiological health status of ruminants and contribute to the diagnosis and prognosis of certain diseases [24,31].

In the present study, WBC, Lym, Mon, and granulocyte (Gran) counts were not affected by zinc supplementation alone in the ZN group. These findings were consistent with previous studies regarding WBC and its subtype counts in lambs [32,33]. However, Aliarabi et al. [34] reported that zinc supplementation (20–40 mg/kg for 18 days) in the diet increased WBC and Lym counts in lambs. Variables such as dosage and duration of supplementation could account for the disparate findings. In the present study, neither NC nor NCZN supplementation altered WBC or its subtype counts in lambs. Similarly, WBC, Lym, Mon, and Gran counts were found to be unaffected by only clinoptilolite supplementation in earlier research on lambs and calves [35,36]. Additionally, ZNRC supplementation did not affect the aforementioned parameters in fattening lambs. Moreover, zinc supplementation alone increased Lym percentages in the ZN group when day 0 and day 42 values were compared. However, zinc supplementation alone did not differ significantly from the C group at day 42. These data were consistent with previous studies [33,37]. In contrast, combined supplementation of Zn and clinoptilolite (NCZN) increased Lym percentages in lambs, whereas clinoptilolite alone (NC) had no effect on Lym percentages. Interestingly, ZNRC supplementation was associated with decreased Lym percentages in the present study. In addition, Mon and Gran percentages were not influenced by the different supplementations in our study. To date, no comprehensive studies have specifically examined the effects of clinoptilolite supplementation on Lym, neutrophil, eosinophil, Mon, and basophil ratios in fattening lambs. It may be suggested that Zn adsorption onto the clinoptilolite surface (ZNRC) could have influenced its ion-exchange capacity, potentially resulting in altered Zn release within the organism.

Although RBC values tended to decrease in the ZN group when day 0 and day 42 were compared, no significant differences were detected among the experimental groups at day 42. Previous studies have reported that Zn supplementation in the diet had no effect on RBC values in lambs [32–34]. Insufficient or excessive

Table 2. Hematological parameters in the different experimental groups at day 0 and day 42.

Parameters		**Trial groups**				
		C	**NC**	**ZN**	**NCZN**	**ZNRC**
WBC ($\times 10^9$/L)	Day 0 Day 42	8.36 ± 0.25 8.55 ± 0.30	8.08 ± 0.23 8.28 ± 0.35	7.85 ± 0.16 8.28 ± 0.13	8.42 ± 0.18 8.53 ± 0.16	7.98 ± 0.23 7.92 ± 0.31
Lym ($\times 10^9$/L)	Day 0 Day 42	85.65 ± 1.33 83.72 ± 1.82	85.97 ± 1.17 84.36 ± 2.18	87.28 ± 1.07 86.21 ± 0.87	85.17 ± 0.97 85.20 ± 0.92	87.03 ± 1.42 86.82 ± 2.32
Mon ($\times 10^9$/L)	Day 0 Day 42	3.15 ± 0.28 3.01 ± 0.29	2.78 ± 0.29 2.91 ± 0.34	2.57 ± 0.16 2.90 ± 0.20	3.15 ± 0.20 3.11 ± 0.13	2.66 ± 0.25 2.46 ± 0.35
Gran ($\times 10^9$/L)	Day 0 Day 42	11.20 ± 1.06 13.26 ± 1.55	11.23 ± 0.90 12.72 ± 1.86	10.13 ± 0.96 10.88 ± 0.72	11.67 ± 0.79 11.68 ± 0.82	10.30 ± 1.22 10.71 ± 1.99
Lym (%)	Day 0 Day 42	72.23 ± 1.26 71.60 ± 1.27**ab**	69.79 ± 1.17 69.84 ± 1.46**ab**	68.93 ± 0.89**B** 71.78 ± 0.68**Aab**	71.91 ± 0.98 73.10 ± 0.90**a**	69.79 ± 1.09 68.75 ± 1.01**b**
Mon (%)	Day 0 Day 42	2.71 ± 0.30 2.64 ± 0.33	2.31 ± 0.31 2.50 ± 0.37	2.04 ± 0.17**B** 2.42 ± 0.19**A**	2.68 ± 0.22 2.68 ± 0.16	2.17 ± 0.27 2.02 ± 0.37
Gran (%)	Day 0 Day 42	9.66 ± 1.09 11.71 ± 1.70	9.27 ± 0.98 11.06 ± 1.98	8.10 ± 0.90 9.13 ± 0.71	9.97 ± 0.82 10.12 ± 0.85	8.46 ± 1.25 8.97 ± 2.02
RBC ($\times 10^{12}$/L)	Day 0 Day 42	11.47 ± 0.25 11.30 ± 0.39	12.11 ± 0.46 11.35 ± 0.29	11.76 ± 0.20**A** 11.14 ± 0.23**B**	11.93 ± 0.24**A** 11.23 ± 0.26**B**	11.45 ± 0.28 11.04 ± 0.15
MCV (fL)	Day 0 Day 42	28.38 ± 0.53 28.65 ± 0.40	28.10 ± 0.44 28.47 ± 0.39	28.18 ± 0.32 28.41 ± 0.33	29.06 ± 0.31 29.42 ± 0.44	28.37 ± 0.44 29.65 ± 0.59
HCT (%)	Day 0 Day 42	32.48 ± 0.77 32.23 ± 0.27	34.00 ± 1.34 32.27 ± 0.92	33.07 ± 0.59**A** 31.63 ± 0.90**B**	34.61 ± 0.79 32.75 ± 0.84	32.47 ± 1.02 32.70 ± 0.73
MCH (pg)	Day 0 Day 42	11.14 ± 0.17**A** 10.03 ± 0.12**B**	11.25 ± 0.10**A** 10.11 ± 0.19**B**	11.32 ± 0.12**A** 10.18 ± 0.11**B**	11.53 ± 0.12**A** 10.18 ± 0.11**B**	11.32 ± 0.17**A** 10.20 ± 0.16**B**
MCHC (g/dL)	Day 0 Day 42	39.72 ± 0.69**A** 35.21 ± 0.28**B**	40.23 ± 0.47**A** 35.65 ± 0.45**B**	40.36 ± 0.43**A** 36.10 ± 0.41**B**	39.91 ± 0.21**A** 35.12 ± 0.51**B**	40.11 ± 0.36**A** 35.73 ± 0.72**B**
HGB (g/dL)	Day 0 Day 42	12.91 ± 0.34**A** 11.37 ± 0.31**B**	13.68 ± 0.54**A** 11.53 ± 0.41**B**	13.36 ± 0.24**A** 11.43 ± 0.34**B**	13.82 ± 0.30**A** 11.51 ± 0.30**B**	13.02 ± 0.36**A** 11.68 ± 0.26**B**
PLT (10^9/L)	Day 0 Day 42	292.87 ± 19.90 284.12 ± 22.87	334.62 ± 53.36 276.00 ± 43.97	265.12 ± 25.78 296.25 ± 21.19	273.75 ± 21.17 253.50 ± 36.98	311.37 ± 29.54 300.25 ± 45.79

Different lowercase letters (a–b) within the same column indicate significant differences between groups ($p < 0.05$), whereas different uppercase letters (A–B) indicate significant differences between sampling periods (day 0 and day 42) ($p < 0.05$). Group C (control): Lamb raising feed only. NC group: Lamb raising feed supplemented with natural clinoptilolite. ZN group: Lamb raising feed supplemented with zinc. NCZN group: Lamb raising feed supplemented with natural clinoptilolite and zinc. ZNRC group: Lamb raising feed supplemented with zinc-reinforced clinoptilolite.

Table 3. Serum IgG concentrations (g/L) in the different experimental groups.

Groups	*n*	**Mean ± SEM** **Day 0**	**Day 42**
C	8	0.980 ± 0.01	0.955 ± 0.013**c**
NC	8	0.975 ± 0.05	0.980 ± 0.018**bc**
ZN	8	0.972 ± 0.04**B**	1.010 ± 0.015**Aab**
NCZN	8	0.968 ± 0.02**B**	1.010 ± 0.011**Aab**
ZNRC	8	0.975 ± 0.01**B**	1.04 ± 0.009**Aa**

Different lowercase letters (a–c) within the same column indicate significant differences among groups ($p < 0.05$), whereas different uppercase letters (A–B) indicate significant differences between sampling periods (day 0 and day 42) ($p < 0.05$). Group C (control): Lamb raising feed only. NC group: Lamb raising feed supplemented with natural clinoptilolite. ZN group: Lamb raising feed supplemented with zinc. NCZN group: Lamb raising feed supplemented with natural clinoptilolite and zinc. ZNRC group: Lamb raising feed supplemented with zinc-reinforced clinoptilolite. SEM: The standard error of the mean.

Table 4. ANAE-positive lymphocyte ratios in the different experimental groups at day 0 and day 42.

Parameters		**Groups** C	 NC	 ZN	 NCZN	 ZNRC
ANAE + lymphocyte	Day 0	71.33 ± 3.03	71.31 ± 1.02	70.22 ± 2.09	71.32 ± 3.02	70.33 ± 2.03
	Day 42	72.14 ± 2.87	71.06 ± 1.79	72.11 ± 2.84	71.16 ± 1.89	73.15 ± 3.88

Group C (control): Lamb raising feed only. NC group: Lamb raising feed supplemented with natural clinoptilolite. ZN group: Lamb raising feed supplemented with zinc. NCZN group; Lamb raising feed supplemented with natural clinoptilolite and zinc. ZNRC group: Lamb raising feed supplemented with zinc-reinforced clinoptilolite.

Zn levels may disrupt RBC formation either through impaired cell division or by inducing oxidative stress in animals [16]. Similarly, NCZN supplementation resulted in RBC value alterations comparable to those observed with ZN supplementation. Additionally, no changes in RBC values were detected among the groups receiving different supplementations at day 42. Previous studies have indicated that clinoptilolite supplementation does not significantly affect RBC values in ruminants [35,36,38]. Furthermore, zinc supplementation alone did not affect MCV values in lambs in the present study. Sobhanirad et al. [39] reported elevated MCV values in lambs receiving 50 mg Zn/kg as zinc propionate for 60 days. Moreover, clinoptilolite supplementation has been reported to increase MCV values in lambs [35] but not in calves [36]. This discrepancy may be attributed to differences in dosage regimens or animal species.

In the present study, HGB, MCH, and MCHC values decreased in the ZN group when day 0 and day 42 were compared. Additionally, these parameters were similar among the groups (C, ZN, NC, NCZN, and ZNRC) at day 42. In contrast, Sobhanirad et al. [39] reported increased HGB, MCH, and MCHC levels in Baluchi fattening lambs supplemented with 100 mg/kg zinc propionate for 60 days. Similarly, Önder and Keçeci [32] demonstrated that supplementation with 40 mg/kg zinc for 6 months increased HGB and MCH values during the 2nd and 3rd months of the study, whereas MCHC values remained unchanged. Reduced physical activity, a common consequence of intensive husbandry, has been reported to exacerbate oxidative stress and impair tissue oxygenation, thereby influencing erythrocyte turnover and function [40]. Although NC, NCZN, and ZNRC supplementations were associated with decreased HGB, MCH, and MCHC values when day 0 and day 42 were compared, no significant differences were detected among the groups at day 42. Consistent with previous studies in ruminants, clinoptilolite supplementation did not affect the aforementioned parameters [35,36,38]. In the present study, HCT values in the ZN group decreased at day 42 compared with day 0. Furthermore, HCT values in the ZN group did not differ significantly from those of the other groups at day 42. These findings were consistent with those reported by Aliarabi et al. [34]. Conversely, Önder and Keçeci [32] reported that HCT values in Konya Merino lambs increased only during the 3rd month of the trial when 250 mg/kg zinc was supplemented for 6 months. These differences appear to depend on the duration of supplementation and the zinc dose. In the present study, NC, NCZN, and/or ZNRC supplementation did not affect HCT values in lambs. These results were consistent with previous studies [35,36,38]. It may be suggested that NC, NCZN, and/or ZNRC supplementation did not disrupt gastrointestinal mineral homeostasis. No changes in PLT values were detected among the experimental groups in the present study. Zengin et al. [37] reported no significant differences in PLT values among experimental groups before (day 0) and after (day 56) supplementation with zinc-enriched yeast. Similarly, Sobhanirad et al. [39] reported that PLT values in Baluchi lambs decreased with 50 mg/kg zinc propionate supplementation but increased with 100 mg/kg supplementation. These findings suggest that PLT values in lambs may be influenced by the dose and duration of zinc administration. Based on group and age averages, clinoptilolite supplementation at 1.5% and 3% levels did not alter fibrinogen values in newborn lambs [35]. These findings regarding PLT values were consistent with a study investigating the effects of short-term clinoptilolite supplementation in colostrum of dairy calves [36]. The hemostatic efficacy of clinoptilolite appears to be context-dependent, being evident in topical applications but limited in dietary supplementation scenarios [41]. Moreover, zinc adsorption onto the clinoptilolite surface did not appear to enhance its hemostatic effect.

Antibodies, also known as immunoglobulins (Ig), are glycoproteins produced by plasma cells. Certain immunogens, such as bacterial proteins, stimulate B cells to differentiate into plasma cells. Plasma cells produce immunoglobulins and participate in humoral immune responses against various antigens, including chemicals, synthetic compounds, bacteria, viruses, fungi, and parasites [18–20]. In the present study, IgG levels increased in the ZN group at day 42 compared with day 0. Similarly, Alijani et al. [42] reported that sheep fed zinc methionine or nano-ZnO exhibited higher blood IgG levels than control animals, with the nano-ZnO group demonstrating greater IgG levels than the conventional ZnO group. Mansour et al. [43] evaluated the effects of nano-ZnO supplementation in lambs with zinc deficiency. The results of that study showed that gamma globulin (γ2, γ1) and beta globulin (β2, β1) levels were higher in the nano-ZnO group compared with the ZnO and control groups. These findings may reflect a humoral immunomodulatory effect of Zn [44]. In the present study, NC supplementation did not affect the IgG levels in fattening lambs. In contrast, Burçak and Yalçın [45] reported that sepiolite supplementation at 1% and 2% for 68 days increased serum IgG levels in lambs. Furthermore, IgG levels were reported to be elevated in studies involving calves fed clinoptilolite as a dietary supplement [18,46,47]. Additionally, NCZN and ZNRC supplementations increased IgG levels in fattening lambs. Their combined immunomodulatory, adsorptive, and ion-exchange properties may improve intestinal health and antigen presentation, which in turn could promote more effective immune responses [16].

ANAE has been proposed as a useful marker for identifying T and B lymphocytes in peripheral blood samples. Furthermore, the proportion of these cells in peripheral blood provides insight into the immune status of the living organism [21]. In the present study, ANAE-positive lymphocyte ratios in fattening lambs were not affected by zinc supplementation alone. Dönmez and Keskin [48] similarly reported that T and B lymphocyte ratios in both groups (control and 250 ppm zinc-supplemented) were comparable throughout the 6 month study in Angora goats. To date, no studies have examined the effect of dietary zinc supplementation on ANAE-positive lymphocyte ratios in other ruminants. While zinc deficiency impairs thymus function and T-cell maturation, optimal Zn levels promote B-cell proliferation and immunoglobulin synthesis. Based on this information, Zn supplementation in the present study appears to have been sufficient and balanced [49]. Conversely, neither NC supplementation alone nor NCZN supplementation altered ANAE-positive lymphocyte ratios in lambs. Additionally, ZNRC supplementation did not produce significant changes in ANAE-positive lymphocyte ratios. Moreover, Eryavuz et al. [50] reported that administration of zeolite (3% clinoptilolite for 30 days) increased plasma tumor necrosis factor-alpha (TNF-α) levels, decreased interleukin-1-beta (IL-1β) levels, and did not affect IL-6 levels in sheep. Jarosz et al. [51] reported that both zeolite-treated groups exhibited higher percentages of $CD4^+CD25^+$ T and B lymphocytes, whereas only the group receiving 3% zeolite showed increased percentages of $CD8^+CD25^+$ T cells. Therefore, the combination of Zn and zeolite (ZNRC) may provide complementary immunomodulatory effects, with Zn directly influencing lymphocyte enzyme activity and zeolite modulating the inflammatory milieu.

5. Conclusion

Although clinoptilolite used as a feed additive did not appear to adversely affect lamb health, its potential positive effects on certain hematological and immunological parameters could not be clearly demonstrated. However, zinc-containing combinations (NCZN and ZNRC) appeared to exert more pronounced effects, particularly with respect to Lym percentages and IgG levels. These findings suggest that alternative strategies may be developed by utilizing the ion-exchange capacity of clinoptilolite. Future studies should incorporate dose–response designs, investigate underlying molecular and cellular mechanisms, and assess long-term health and productivity outcomes to further elucidate the potential of ZNRC supplementation as a nutritional strategy for enhancing immune competence and overall animal health.

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Conflict of interest

The authors declare no conflicts of interest.

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Author contributions

İhsan Kısadere conducted the experiments, processed the samples, analyzed the data, and drafted the manuscript; this author also serves as the corresponding author. Muhittin Zengin and Özkan Demirbaş conceived and designed the study, supervised the project, contributed to conceptualization, assisted in interpreting the results, and critically revised the manuscript. Mehmet Faruk Aydın served on the advisory committee, provided methodological input, and reviewed the manuscript. All authors have read and approved the final manuscript and agree to be accountable for the integrity of the work.

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