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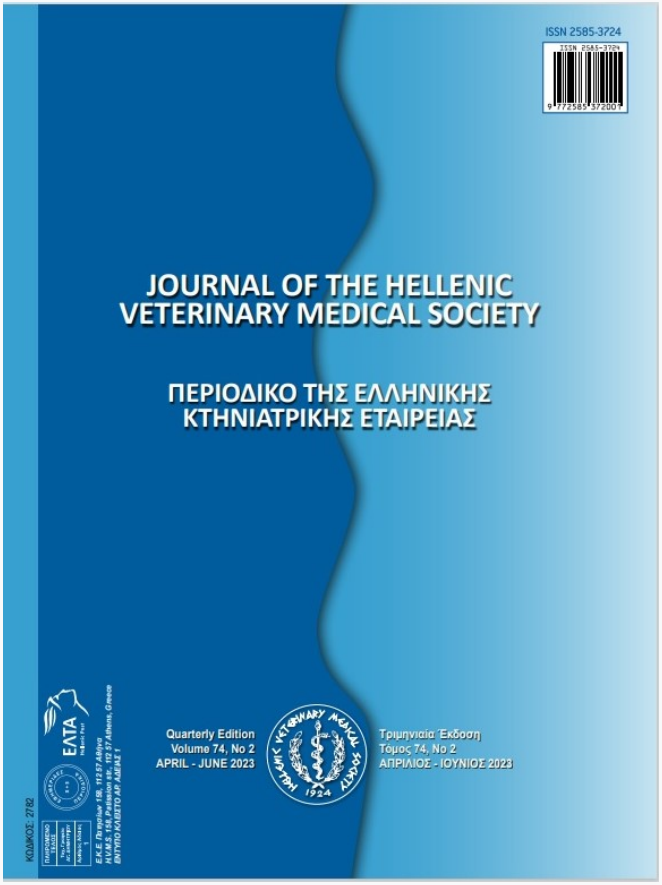

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Research article
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Effect of Some Immunomodulators Use Together with PPR Vaccine on Immune system in Morkaraman Sheep

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ABSTRACT: In this study, it was aimed to show the effects of vitamin C (Vit-C), vitamin D (Vit-D), levamisole (LMS), inactivated *parapoxvirus ovis* (iPPVO) and *corynebacterium cutis lysate* (CCL) on the immune system in Morkaraman sheep on which the peste des petitis ruminant (PPR) vaccine was administered. In the Group B (Vit-D + PPR vaccine), in terms of IgG, when compared 0th (1.27±0.15), 14th (1.08±.0.21), 21st (1.23±0.23) and 28th (1.26±0.42), the values of the day 7th (1.68±0.24) were determined to be significantly higher (P<0.01). In comparison to overall evaluation based on groups of IgG assay, the Group C (LMS + PPR vaccine) value (1.79±1.18) was determined to be significantly higher than the Group F (PPR vaccine) (1.21±0.43), Group A (Vit-C + PPR vaccine) (1.27±0.51), Group B (1.31±0.33) and Group D (iPPVO + PPR vaccine) values (1.38±0.48) (P<0.01). In terms of IgM, when days among groups are compared, the highest value was determined on the 7th day in the Group A (0.50±0.13) and this value was determined to be significant according to the 7th day Group F (0.28±0.11), Group E (CCL + PPR vaccine) (0.30±0.05) and Group D values (0.24±0.03) (P<0.01). In comparison to overall evaluation based on groups of IgM assay, the Group A value (0.45±0.15) was determined to be significantly higher than the Group D (0.33±0.14), Group E (0.34±0.19), Group F values (0.33±0.22) (P<0.05). In the comparison of group-based general evaluations of globulin values, it was determined that the values obtained in the Group A (4.33±0.55), Group B (4.33±0.48), Group C (4.20±0.50), Group D (4.24±0.33) and Group E (4.29±0.29) groups were significantly higher than the Group F (4.00±0.33) (P<0.01). It was concluded that the application of LMS at the stated dose had positive effects on the immune system due to changes in both IgM and IgG values, and Vit-C application at the stated dose had a partially positive effect only due to changes in IgM value; the administration of Vit-D, iPPVO and CCL at the stated doses did not have any positive effect on the immune system.

Keywords: Immune System; Immunostimulant; Peste Des Petitis Ruminant Vaccine.

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INTRODUCTION

All living species, from the simplest to the most complex organisms, have the ability to recognize familiar species coming from the same family and distinguish the foreign ones. Many cells, tissues, systems and organs are involved in the immune response processes, which starts with the entry of these foreign substances, defined as antigen (Ag), and is shaped by many of reactions that have very close relations with each other. The organs and different cells that have a role in these complex events are called the immune system (Bilgehan, 1993).

The immune system is classified as hereditary and acquired immune system (Daha, 2011). Hereditary immune system cells include natural killer cells, mast cells, basophils, eosinophils, macrophages, neutrophils and phagocytic cells including dendritic cells (Gasque, 2004). The acquired immune system is comprised of T and B lymphocytes (Fine et al., 1994). The most negatively charged part of serum proteins is albumin. Other serum proteins consist of alpha, beta and gamma globulins (γ-globulin). Immunoglobulin M (IgM) is transported by beta globulins, while a great number of immunoglobulins (Ig) exist in γ-globulin and have (γ1-globulin % 18.9 ± 2.8; γ2-globulin % 3.6 ± 1.0) in serum (Tizard, 2013; Nagy et al., 2015). Immune response is mainly induced by Igs or antibodies (Abs) (Mix et al., 2006). Immunoglobulin G (IgG) is the one that has the highest level (70-80%) in the blood (Diker, 2005). They pass through blood vessels more easily than other Igs because they are the smallest ones in size compared to others especially smallest than IgM (Tizard, 2013). IgM are associated with a primary immune response and are often used to diagnose acute exposure to an immunogen or pathogen (Boes, 2000). Immunstimulants are substances that stimulate the immune system of animals against various bacterial and viral diseases. It stimulates the immune system non-specifically against the antigen. They can be used alone to increase resistance to diseases, as well as in combination with antibacterial, antiviral or antiparasitic drugs specific to the type of disease (Aydın and Aktaş, 2021).

The purpose of this study is to comparatively examine the effects of Vitamin C (Vit-C), Vitamin D (Vit-D), Levamisole (LMS), Inactive *Parapoxvirus ovis* (iPPVO) and *Corynebacterium Cutis Lysate* (CCL) on the immune system of Morkaraman sheep vaccinated by peste des petits ruminants vaccine was administered (PPR).

MATERIALS AND METHODS

Animals

This study was carried out in the Sheep Breeding Unit of Ataturk University Food and Livestock Application and Research Center Directorate in Erzurum. 48 Morkaraman sheep, which were aged between 2 to 4 years old (average 3,2), healthy, female and were subjected to the same care and feeding conditions were used within the scope of the study. Before starting the study, all sheep were given an antiparasitic drug (Ivomec®, Merial, France) containing 10 mg Ivermectin per 1 mL 1 cc/50 kg single dose and 100,000 IU benzylpenicillin benzathine, 100,000 IU Benzyl penicillin-Procaine and 250 mg Dihydrostreptomycin sulfate in 1 mL an effective antibiotic (Hipracilin Retard®, Hipra, Spain) was administered in a single dose of 1 cc/20 kg. Antiparasitic and antibiotic (Hipracilin Retard®, was administered in a single dose of 1 cc/20 kg) administrations were applied to prevent possible secondary infections, and one week after these applications, clinical examination (body temperature, pulse, respiration, examination of palpable lymph nodes and diarrhea, blood and constipation in the stool) was performed on sheep and healthy ones were included in the study.

Study design

Group A (Vit-C group, n = 8): 40 mg/kg single dose of Vit-C (Vitce®, Verano, Turkey) containing 200 mg of ascorbic acid in 1 mL as well as PPR vaccine was subcutaneously administered to the sheep in this group (Roth and Kaeberle, 1985).

Group B (Vit-D grubu, n= 8): 6000 IU/kg single dose of Vit-D (Egevet-D3® Ege-Vet, the Netherlands) containing 1.000.000 IU cholecalciferol (Vitamin D3) in 1 mL as well as PPR vaccine was intramuscularly administered to the sheep in this group (Smith and Wright, 1985).

Group C (LMS group, n= 8): LMS containing LMS HCl (Levamis %10®, Provet, Turkey) which is equivalent to 100 mg LMS in 1 mL was subcutaneously administered to sheep in this group at a dose of 5 mg/kg for 4 days as well as PPR vaccine (Undiandeye et al., 2014).

Group D (iPPVO group, n = 8): IPPVO (Zylexis, Zoetis, Belgium) that contains at least 230 units of interferon iPPVO D 1701 strain in 1 mL was intramuscularly administered at a dose of 2 ml/sheep twice with 3 days intervals together with PPR vaccine (Gokce et al., 1997).

Group E (CCL group, n = 8): 1 ml/ sheep of CCL (Ultracorn®, Virbac, France) containing 20 mg CCL in 1 mL was subcutaneously administered to the sheep in this group as well as PPR vaccine (Dik et al., 2016).

Group F (Control group, n = 8): Only PPR vaccine containing attenuated PPR 75/1 $10^{2.5}$ $DKID_{50}$/ dose was administered to sheep in this group (Pest-S Etvac®, Etlik Veterinary Control Central Research Institute, Turkey).

Sample collection

Blood samples taken from *V. Jugularis* of the sheep in all groups were added to 10 mL serum tubes (Becton Dickinson Co., USA) and tubes with EDTA (ethylenediaminetetraacetic acid) on 0 (pre-trial), 7, 14, 21 and 28 days. (Becton Dickinson Co., USA). The blood samples taken into serum tubes were kept at room temperature for 30 minutes to separate the serum and the serum tubes were centrifuged at 3000 rpm for 15 minutes. Serum samples were kept at -80 °C until analysis. Hematological analyzes were performed immediately in blood samples taken into tubes with EDTA (3.6 mg K_2E, BD Vacutainer, BD-Plymouth, UK., 2ml).

Hematological, immunological and biochemical analyses

Hematological analyzes of the blood samples taken were performed using a hemogram device. Total leukocyte (WBC), lymphocyte (LYM), monocyte (MON), neutrophil (NEU) counts were determined and recorded in the analyzes (Abacus Junior Vet-5®, Austria). IgG and IgM levels in serum samples were measured using sheep specific ELISA test kit (IgM, catalog no: YLA0006SH, IgG, catalog no: YLA0003SH, YL biont, China). The analyzes were performed in line with the manufacturer's recommendations. Total protein and albumin levels in serum samples were measured using an autoanalyzer (Beckman Coulter AU5800, USA). The globulin value was obtained by subtracting the albumin value from the total protein value (Roussel et al.,1997; Gokce et al., 2007).

Ethical approval

The ethical consent from the local ethical committee of animal experiments in Ataturk University was also obtained in (Decision Number: 2018/54).

Statistical analysis

General Linear Model procedure was used in the analysis of variance in the comparison of the hematological, immunological and biochemical parameters within each sheep in the group based on days, and the general averages of the groups. The significance checks of the data deemed important were made using the SPSS statistical computer program (version 20). Differences between groups were determined by Duncan multiple comparison test ($P < 0.05$).

RESULTS

Clinical findings

No pathological findings (body temperature, pulse, respiration, and examination of palpable lymph nodes, presence of diarrhea, blood and constipation) were observed during the clinical examination of the sheep in this study.

Hematological, immunological and biochemical findings

Hematological, immunological and biochemical data obtained from blood samples are shown in tables 1, 2, 3 and 4. In the general evaluation inter-groups, the lowest value was obtained in Group B and this value was found to be significant ($P<0.01$) (Table 1). At the same time, there was a considerable difference between Group B and C in the general evaluation inter-groups ($P< 0.01$) (Table 2). In the comparison of intra-group days in terms of IgG values, the 7th day value for Group B was higher than the other days of the study, and this value was found to be important ($P<0.01$). In the general inter-groups evaluation, the numerically highest value was obtained in Group C and significant differences were obtained between Group C, A, B, D, F values ($P<0.01$). While no significant differences were obtained within groups for IgM values, it was determined that the highest numerical value was in Group A and significant differences were formed between Group A, D, E, F ($P<0.01$). There was a significant difference between the Group B and C with the Group D and F values in terms of IgM values in the inter-groups comparison ($P< 0.01$). In the general inter-groups assessment, the highest IgM value was obtained in Group A, and there was a significant difference between Group A, D, E, and F ($P<0.05$). Similarly a significant difference was also achieved between Group C and D, Group E and F ($P< 0.05$) (Table 3). In the general inter-groups evaluation for globulin values, the lowest numerical value was found in Group F, and a significant difference was determined between the values of the experimented groups and the value of the control group (Group F) ($P<0.01$) (Table 4)

Table 1. The number of mean total leukocyte and lymphocyte of the groups, statistical comparisons within and between groups.

Groups								
Parameters	Day	Group A X̄±SD	Group B X̄±SD	Group C X̄±SD	Group D X̄±SD	Group E X̄±SD	Group F X̄±SD	P
Total leukocyte count (×10³ µL)	0	10.43±3.87	8.13±2.78	9.44±3,69	11.85±3.80	9.82±2.70	10.02±1.47	>0.05
	7	10.01±3.77	8.63±1.88	9.58±1.81	10.82±2.29	10.86±3.44	10.64±1.19	>0.05
	14	9.76±3.11	8.05±1.95	10.07±2.03	10.94±2.10	9.49±2.43	10.25±0,91	>0.05
	21	11.05±4.63	9.01±2.32	11.29±2.25	11.34±2.22	10.05±3.60	10.98±1.38	>0.05
	28	10.58±3.61	9.16±1.65	12.06±4.25	10.50±2.07	10.79±3.04	10.25±2.19	>0.05
	P	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	
Overall evaluation based on groups		10.36±3.65^{b}	8.59±2.09^{a}	10.49±3.00^{b}	11.09±2.49^{b}	10.20±2.96^{b}	10.43±1.45^{b}	**<0.01**
Lymphocyte count (×10³/µL)	0	8.18±3.04	5.67±2.16	6.54±2.39	7.96±2.42	8.38±1.50	6.90±0.78	>0.05
	7	6.35±2.12	5.38±1.58	6.42±1.27	6.94±1.39	7.36±2.53	7.01±0.89	>0.05
	14	6.20±1.86	5.20±1.51	6.63±1.13	6.77±1.41	6.56±1.62	6.94±0.88	>0.05
	21	6.89±2.82	5.71±1.53	7.05±1.12	7.11±1.26	6.49±2.25	7.31±1.24	>0.05
	28	6.61±2.28	6.10±1.44	7.53±2.29	6.73±1.31	7.05±1.49	6.57±1.62	>0.05
	P	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	
Overall evaluation based on groups		6.85±2.44^{b}	5.61±1.61^{a}	6.83±1.70^{b}	7.10±1.60^{b}	7.17±1.95^{b}	6.95±1.09^{b}	**<0.01**

Different capital letters in the same column indicate the statistical difference between the days in the group. The different lowercase letters in the same line indicate the statistical difference in overall assessment within groups and between groups on the same day

Table 2. The number of mean monocyte and neutrophil of the groups, statistical comparisons within and between groups.

Groups								
Parameters	Day	Group A X̄±SD	Group B X̄±SD	Group C X̄±SD	Group D X̄±SD	Group E X̄±SD	Group F X̄±SD	P
Monocyte count (×10³/µL)	0	0.05±0.01	0.04±0.01	0.04±0.01	0.05±0.01	0.05±0.01	0.04±0.01	>0.05
	7	0.05±0.01	0.04±0.01	0.04±0.01	0.05±0.01	0.05±0.01	0.05±0.01	>0.05
	14	0.04±0.01	0.04±0.01	0.05±0.01	0.05±0.01	0.04±0.01	0.05±0.01	>0.05
	21	0.05±0.02	0.04±0.01	0.05±0.01	0.05±0.01	0.05±0.01	0.05±0.01	>0.05
	28	0.05±0.01	0.04±0.01	0.06±0.02	0.05±0.01	0.05±0.01	0.05±0.01	>0.05
	P	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	
Overall evaluation based on groups		0.05±0.02^{b}	0.04±0.01^{a}	0.05±0.02^{b}	0.06±0.01^{b}	0.05±0.01^{b}	0.05±0.01^{b}	**P<0.01**
Neutrophil count (×10³/µL)	0	2.20±1.01	2.41±0.94	2.85±1.35	3.84±2.20	2.04±0.86	3.07±0.79	>0.05
	7	3.61±1.71	3.20±0.50	3.11±0.87	3.82±1.12	3.43±1.03	3.58±0.72	>0.05
	14	3.50±1.37	2.59±0.51	3.39±1.23	4.12±1.34	2.88±1.01	3,26±0,75	>0.05
	21	4.10±1.86	3.25±0.99	4.18±1.34	4.17±1.20	3.51±1.44	3.60±0,92	>0.05
	28	3.90±1.41	3.01±0.73	4.47±2.07	3.71±1.11	3.68±1.78	3.16±0.83	>0.05
	P	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	
Overall evaluation based on groups		3.47±1.58^{ab}	2.90±0.80^{a}	3.60±1.50^{bc}	3.94±1.40^{c}	3.11±1.35^{ab}	3.34±0.80^{ab}	**P<0.01**

Different capital letters in the same column indicate the statistical difference between the days in the group. The different lowercase letters in the same line indicate the statistical difference in overall assessment within groups and between groups on the same day.

Table 3. The values of mean IgG and IgM of the groups, statistical comparisons within and between groups.

Groups								
Parameters	Day	Group A X̄±SD	Group B X̄±SD	Group C X̄±SD	Group D X±SD	Group E X̄±SD	Group F X±SD	P
IgG (mg/mL)	0	0.89±0.20	1.27±0.15^{A}	1.54±0.34	1.64±0.66	1.56±0.97	1.53±0.31	>0.05
	7	1.31±0.68	1.68±0.24^{B}	1.85±1.31	1.40±0.43	1.31±0.27	1.22±0.37	>0.05
	14	1.34±0.49	1.08±.0.21^{A}	1.69±1.22	1.29±0.49	1.31±0.65	1.07±0.33	>0.05
	21	1.27±0.38	1.23±0.23^{A}	1.86±1.39	1.39±0.35	1.69±1.16	1.06±0.62	>0.05
	28	1.51±0.57	1.26±0.42^{A}	1.97±1.55	1.13±0.36	1.83±1.01	1.16±0.36	>0.05
	P	>0.05	**<0.01**	>0.05	>0.05	>0.05	>0.05	
Overall evaluation based on groups		1.27±0.51^{a}	1.31±0.33^{a}	1.79±1.18^{b}	1.38±0.48^{a}	1.55±0.85^{ab}	1.21±0.43^{a}	**P<0.01**
IgM (mg/mL)	0	0.42±0.09	0.46±0.20	0.46±0.26	0.31±0.08	0.43±0.31	0.24±0.06	>0.05
	7	0.50±0.13^{c}	0.40±0.14^{bc}	0.39±0.06^{bc}	0.24±0.03^{a}	0.30±0.05^{ab}	0.28±0.11^{a}	**<0.01**
	14	0.41±0.15	0.30±0.09	0.53±0.29	0.36±0.09	0.29±0.09	0.32±0.15	>0.05
	21	0.48±0.21	0.48±0.44	0.42±0.06	0.43±0.24	0.28±0.07	0.32±0.10	>0.05
	28	0.44±0.13	0.26±0.07	0.33±0.07	0.30±0.10	0.39±0.2	0.47±0.43	>0.05
	P	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	
Overall evaluation based on groups		0.45±0.15^{b}	0.38±0.24^{ab}	0.43±0.18^{b}	0.33±0.14^{a}	0.34±0.19^{a}	0.33±0.22^{a}	**P<0.05**

Different capital letters in the same column indicate the statistical difference between the days in the group.The different lowercase letters in the same line indicate the statistical difference in overall assessment within groups and between groups on the same day.

Table 4. The values of mean globulin of the groups, statistical comparisons within and between groups

Groups								
Parameters	Day	Group A X̄±SD	Group B X̄±SD	Group C X̄±SD	Group D X±SD	Group E X̄±SD	Group F X±SD	P
Globulin (g/dL)	0	4.40± 0.39	4.25±0.44	4.20±0.55	4.11±0.28	4.36±0.30	3.88±0.36	>0.05
	7	4.14±0.26	4.21±0.48	4.09±0.53	4.10±0.35	4.19±0.24	3.90±0.30	>0.05
	14	4.25± 0.50	4.31±0.47	4.19±0.44	4.28±0.30	4.40±0.27	3.98±0.34	>0.05
	21	4.43±0.72	4.44±0.55	4.16±0.56	4.33±0.35	4.24±0.34	4.12±0.34	>0.05
	28	4.45±0.79	4.43±0.51	4.38±0.51	4.33±0.35	4.24±0.30	4.10±0.35	>0.05
	P	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	
Overall evaluation based on groups		4.33±0.55^{b}	4.33±0.48^{b}	4.20±0.50^{b}	4.24±0.33^{b}	4.29±0.29^{b}	4.00±0.33^{a}	**P<0.01**

Different capital letters in the same column indicate the statistical difference between the days in the group.The different lowercase letters in the same line indicate the statistical difference in overall assessment within groups and between groups on the same day.

DISCUSSION

Vitamins are organic components of feed. They are extremely necessary for the continuation of life and good health. In case of deficiency of some vitamins, diseases can also occur. Vitamins play an important role in maintaining the immune system of animals and their resistance to diseases (Rasikh, 2019). Vit-C promotes various cellular functions in the cells of the hereditary and acquired system and therefore stimulates the immune system. Vit-C accumulates in phagocytic cells such as neutrophils and increases their chemotactic response (Carr and Maggini, 2017). A great number of studies have been conducted to investigate the effects of Vit-C on hematological parameters in animals and these studies have revealed different results. These studies can be listed as follows: It has been stated that Vit-C administration to sheep before transplantation causes an increase in erythrocyte and WBC counts (Kassab and Mohammed, 2014). It has been reported that the administration of Vit-C in calves causes a decrease in LYM and MON counts, but it has been stated that there is no biological explanation for this situation and it is a random effect (Seifi et al.,2010). Abejide et al. (2013) stated that there was no difference in WBC and NEU counts in sheep, and the number of LYM increased, Hesta et al. (2009) reported that there was no change in hematological parameters in dogs. The current study revealed that there

was no significant difference in hematological parameters within the Group A and F (P>0.05). Animals in Group A were with normal extent of body temperature, pulse, respiration, and palpable lymph nodes. It is known that Vit-C effects on various components of the immune system; some of these effects stimulate both the production (Jariwalla and Harakeh, 1996) and the function (Levy et al., 1996) of leukocytes, especially neutrophils, lymphocytes and phagocytes. Although the data obtained from the study partially overlap with this information, they also differ considerably. The possible reasons are the pharmacokinetics of Vit-C, the inability to understand the needs of the living creature and the difference in the designs of the studies that have been conducted on the subject so far.

Blair and Cummins (1984) suggested that oral administration of Vit-C in calves caused an increase in IgG concentration. Cummins and Brunner (1991) argued that plasma IgG levels increased in parallel with the increase in plasma Vit-C in calves kept in metal shelters. Tyler and Cummins (2003) reported that IgG levels were within normal limits in groups receiving and not receiving ascorbyl 2 phosphate in Holstein breed cattle. For the IgM value, a significant difference was observed between the Group A and the F on the 7th day (P<0.01). Similarly, in the group-based evaluation, a significant difference was observed between the Group F and the A (P<0.05). In the group-based evaluations for globulin value, there was a significant difference between the Group A and the F (P<0.01). In this study, it is believed that the increase in immunoglobulin values, as expressed by Huijskens et al. (2014), increases in positive production due to the effect of vitamin C and triggers the production of antibodies in parallel with the maturation of immature lymphocytes.

It has been reported that 1,25 dihydroxyvitamin D3 has an antiproliferative property, inhibits interferon gamma, interleukin-2 (IL-2) production, increases interleukin-4, interleukin-5 and interleukin-10 production, and stimulates Ig synthesis (Ametaj et al., 1996). With the administration of Vit-D, Rong et al. (2007) found that the number of MON and WBC increased in cow, Rashid and Yuksek (2019) determined that the number of WBC decreased in sheep. The study revealed no significant difference in the hematological parameters within the Group B, and F (P>0.05). In the comparison of the group-based general evaluations of the hematological data of the Group B and the F, it was observed that the number of WBC, LYM and MON were lower in the Group B, and this decrease was significant (P<0.01). It was thought that this difference is possible to stem from the lower pre-trial values of the Group B compared to the Group F. The data obtained from the study are similar to the studies conducted by von Rosenberg et al. (2016) and Rashid and Yuksek (2019) in the way that they indicate that the administration of Vit-D had no effect on such parameters as WBC, LYM, MON and NEU. In this study, the hemogram data of the Group B demonstrates that it was similar to the studies detailed above, as well as differences. It is possible reason for the occurrence of this situation may be due to the difference in the dose of Vit-D administered, the difference in the subject used in the study and the differences in the existing Vit-D levels in animals.

In studies investigating the effects of Vit-D on Ig parameters, Yue et al. (2018) found that plasma and milk Ig concentrations displayed no change in cattle as a result of Vit-D, Reinhardt et al. (1999) reported that serum IgG, especially IgG1 titers increased in cattle. In this study, a significant increase (P<0.01) was observed in the 7th day value of IgG in the Group B compared to the other days of the study. When the IgM values of the Group B and the F were compared based on days, there was significant difference on the 7th day (P<0.01). In the group-based evaluation in terms of globulin values, there was a significant difference between the Group B and the F (P<0.01). It is possible to state that the reason for the change in IgG and IgM values may stem from the fact that Vit-D activates the humoral immune system rather than the cellular immune system as Rashid and Yuksek (2019).

Levamisole is an immunomodulator and its immunostimulants properties become apparent and prominent when used at a repetitive dose of 2.5 mg/kg before vaccination in different animal species (Stelletta et al., 2004). Abdullah and Basbugan (2020) found that the number of WBC decreased in sheep LMS group, Rashid and Yuksek (2019) stated that a statistically significant decrease was observed in the numbers of WBC and LYM in the group in which LMS was used with the enterotoxemia vaccine in sheep. In this study, when the number of WBC within the Group C was compared between the days, it was determined that there was no statistically significant difference (P>0.05), but a numerical increase was found in WBC count. The same situation was also seen in LYM and NEU counts (P>0.05). Possible causes of numerical changes of the Group C are that LMS has

an cholinergic effect on leukocytes, the potential to increase protein, nucleic acid synthesis in passive leukocytes and thus causes an increase in the number of leukocytes as Bourne et al. (1978).

The studies on levamisole administration have shown an overall increase in Ig levels. Abdullah and Basbugan (2020) showed that IgM levels in sheep increased but it was not statistically significant, IgG levels, on the other hand, increased both numerically and gave a statistically significant result. In this study, it was determined that there was a numerical tendency to increase in IgG values within the Group C ($P>0.05$), and a significant numerical increase was observed ($P<0.01$) in the group-based general evaluation compared to the Group F. Comparing the IgM data of the Group C and F, the difference on the 7th day was statistically significant ($P<0.01$), and the comparison of evaluations of group-based regarding the Group C and F indicated that there was a differences between them ($P<0.05$). In addition, in terms of globulin values, there has also significant difference ($P<0.01$). These data obtained show that LMS strengthens the immune system by causing an increase in Ig levels such as IgG and IgM and this time are in accordance with the reports of Abdullah and Basbugan (2020).

Inactive *Parapoxvirus ovis* is used as an immunostimulatory biological agent for the prevention/treatment of infectious diseases in veterinary medicine because of its strong immunomodulatory activity in many species (cow, mouse, guinea pig, horse) (Schutze et al., 2009). Pekmezci et al. (2014) stated that in dogs with demodicosis, iPPVO treatment caused no change in the number of WBC, MON and granulocyte between the groups, and the number of LYM was higher in the iPPVO group than the amitraz group on the 10th day of the study. In this study there was no significant difference in hematological parameters in the Group D compared to the Group F ($P>0.05$). In comparison of the group-based general evaluations of hematological parameters, it was determined that the increase in NEU value was statistically significant in the Group D compared to the Group F ($P<0.01$).

It has been reported that iPPVO increases NEU counts, interferon, and interleukin and tumor necrosis factor production (Greggs, 2013). Therefore, the increase in the number of NEU in this study is in accordance with this report. It has been evaluated that the existence of different results in terms of the effects of iPPVO administration on hematological parameters may have been caused by the differences in the design of the studies, the difference in the dose and administration methods, and it was concluded that more comprehensive studies should be conduct on this subject.

Proksch et al. (2014) found that there was a significant increase in Abs titers in dogs after the use of iPPVO, Schutze et al. (2010) reported that IgG in dogs increased at a statistically significant rate 2 weeks and 2 months after its administration. In order to analyze the IgG and IgM values, the study evaluated the Group D between days and within the group; the study also compared the Group F with D, and also performed an overall group-based evaluation. The evaluation revealed that there was no significant difference between the Group F and the D ($P>0.05$). In the group-based evaluation, it was determined that the Group D value in terms of globulin values, was numerically higher than the Group F, and this increase was significant ($P<0.01$). The data obtained from Igs in the Group D revealed that iPPVO administration with PPR vaccine did not lead to significant changes in IgG and IgM levels.

Corynebacterium cutis lysate is a powerful immunostimulant and has also been reported to activate the immune system when used with a vaccine (Mohamed et al., 2013). It is stated that changes in hematological parameters may be caused by increases and decreases in cytokine levels with CCL administration (Yilmaz and Kasikci, 2013). Er et al. (2015) found that CCL administration did not cause a significant change in hematological parameters in sheep. Dik et al. (2016) determined that the administration of CCL showed a decrease in the numbers of WBC and LYM in sheep. This study is accordance with the study conducted by Er et al. (2015) by similarly concluding that there was no statistically significant difference when animals within the Group E based on days were compared, the comparison of the Group E with the Group F was performed and the overall comparison of group-based general evaluations of hematological parameters was taken into consideration ($P>0.05$).

Yılmaz et al. (2011) argued that the use of CCL led to an increase in the level of IgG in the colostrum and serum of sheep and in newborns, Dik et al. (2016) pointed out that the protective immune response continued throughout the study process in sheep which received combined treatment (CCL and PPR vaccine) although the peak level of Abs in the group started 7 days after administration, In addition, Saat et al. (2016) stated that the IgG ratio in the milk of cattle with mastitis increased significantly after the admin-

istration. There seems no study examining the IgM level by applying CCL. In this study, when the data of the Group E and the Group F were compared in terms of IgG value depending the days there was no statistically significant difference (P>0.05), but it was clearly observed that the value in the Group E numerically increased on 21st and 28th days. There was no significant difference for IgM values in the Group E, when compared with the Group F, within the group, between groups and based on the overall group-based evaluation (P>0.05). In terms of globulin values, it was determined that the Group E value was higher than the Group F, and this high value was significant (P<0.01). CCL has been reported to stimulate Abs synthesis by activating both T and B lymphocytes (Halpern et al., 1973). It has been argued that interleukin-1 (IL-1) either alone or in combination with cytokines such as interleukin-6 (IL-6) and IL-2 increases B lymphocyte proliferation or transforms B lymphocytes into Ag-presenting cells, thus playing a role on Ag-activated B lymphocytes (Vink et al., 1988). Consistent with these reports and statements, the possible reason for numerical changes in IgG values in the Group E was not statistically significant, but the possible reason for the numerical changes was the increase in cytokine synthesis with the effect of CCL administration and the increase in IgG concentration with the effect of IL-1 and IL-6 synthesis in particular.

Rashid and Yuksek (2019) determined that there was no significant difference in Ig parameters of control group over a period of 35 days. In addition, in the study of Gulyaz et al. (2020) which investigates the effects of ecthyma vaccine on immunity against foot and mouth disease in sheep, it was stated that there was no significant difference between the groups that received only vaccine and the combined treatment with vaccine on the 30th and 60th days. The same results were obtained in this 28-day follow-up study, and evaluated that a longer follow-up is required to reveal the differences.

CONCLUSION

The study revealed that 40 mg/kg dose of Vit-C administration together with PPR vaccine was partially effective on the immune system and the administration of Vit-D at a dose of 6000 IU/kg with PPR vaccine, iPPVO at a dose of 2 mL per sheep and CCL at a dose of 1 mL per sheep had no effects on the immune system; LMS administration at a dose of 5 mg/kg/day together with the PPR vaccine is effective on the immune system; The comparison between groups indicated that LMS is more effective on the immune system compared to Vit-C, Vit-D, iPPVO and CCL, and further studies are required to shed light on the effects of the related compounds on the immune system in their administration with vaccines.

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CONFLICT OF INTEREST

The authors declared no conflicts of interest.

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