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ORIGINAL PAPER

EFFECTS OF DIFFERENT DIETS ON HEMATOLOGICAL PARAMETERS AND IMMUNOGLOBULIN LEVELS IN NORDUZ SHEEP

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Abstract

In this study, changes in the hematological parameters and immunoglobulin levels of sheep fed different diets were investigated. Healthy, nonpregnant, female Norduz sheep age 1 - 2 years with live weights of 50 - 55 kg that were raised at the Agricultural Research and Implementation Center of Van Yüzüncü Yıl University constituted the animals used in this study. A total of 40 sheep comprised three groups: a control group (n = 20) and two experimental groups (n = 10 in each group). During daylight hours, the sheep grazed primarily on low-quality pasture and lesser amounts of grass. In addition to pasture grazing, all groups were supplied with 850 g d^{-1} of mixed grass-clover hay per head. Aside from pasture grass and mixed grass-clover hay, the control group animals were also supplied with 50 g d^{-1} of barley per head. Each animal in the experimental group (groups 1 and 2) was also supplied with increasing amounts of barley each week: 65 g d^{-1}, 95 g d^{-1}, 110 g d^{-1}, and 130 g d^{-1} in the first, second, third, and fourth weeks, respectively. Aside from pasture grass, mixed grass-clover hay and barley, animals from the second group were also supplied with 500 g d^{-1} of concentrated feed per head throughout the experiment (4 weeks). This study revealed that improved dietary quality causes significant increases in hematological parameters and immunoglobulin levels. The results showed that the hematocrit levels, mean corpuscular volumes, and mean corpuscular hemoglobin concentration values of group 2 were higher than those of the other groups (P<0.05 for hematocrit; P<0.01 for other comparisons). The diets enriched with the concentrated feed increased the immunoglobulin A, immunoglobulin G, immunoglobulin E, and immunoglobulin M levels in sheep compared with levels in the control group (P<0.01). Improvements in these values will reduce the risk of disease in animals and naturally increase their productivity.

Keywords: sheep, diets, hematological parameters, immunoglobulin concentrations.

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INTRODUCTION

Turkey is one of the most important sheep-producing countries in the world, with 37,276,050 head of sheep; the Van province in the Eastern Anatolian region is the biggest sheep producer (2,505,417 head) in Turkey (Tuik 2019). Norduz sheep are reared on the Norduz plateau in the Gürpınar district of the Van province (Tuncer et al. 2017). Principal characteristics of Norduz sheep are high pre-weaning survival rate of lambs and the capacity for adaptation (Bingöl 1998). In an economical sheep breeding model, animal immune systems must be strong to reduce the risk of disease and prevent deaths.

Determination of blood parameters is important in the diagnosis of animal diseases that directly affect yield characteristics (Rajaian 1993, Bozdoğan et al. 2003). These parameters may vary depending on the animal species, breed, age, gender, regional conditions, and feeding programs (Nisbet et al. 2006). Therefore, determining the blood parameters may aid in animal care, nutrition, disease diagnosis, and disease treatment. (Stephan, Ettinger 1989, Çelebi, Uzun 2000).

Neonatal lamb diseases and mortality constitute the majority of yield losses in sheep breeding operations (Kozat 2019). Lamb mortality and the risk of disease in the neonatal period are mostly related to the insufficient colostrum supply to lambs or the imbalanced composition of colostrum. In sheep breeding, the health of newborn lambs and the lamb's sustained viability are among the most difficult management problems (Koyuncu, Duymaz 2017). Feeding sheep with sufficient and balanced diets in the prenatal period reduces the risk of disease in pregnant sheep and allows newborn lambs to obtain sufficient and balanced colostrum (Nowak, Poindron 2006, Kozat 2019). Feeding with balanced diets strengthens the immune system, especially throughout pregnancy, and improves colostrum composition. Feeding during late pregnancy may increase colostrum production (O'Doherty, Crosby 1997). Immunoglobulins are extremely important to the immune system. Immunoglobulins, or antibodies, are used by the immune system to recognize and neutralize antigens like bacteria and viruses (Nowak, Poindron 2006, Şentürk, Esen 2012). They also play roles in cytokine production and cell adhesion, the deactivation of toxin molecules and viruses, the enrichment of mucosal immunity, the prevention of the attachment of microorganisms onto mucosa, and the prevention of intestinal absorption of harmful molecules (Şentürk, Esen 2012, Yilmaz, Akgül 2014). Immunoglobulins can be classified as immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin E (IgE), and immunoglobulin M (IgM) according to their physical, chemical, and immunological differences (Yilmaz, Akgül 2014). Immunoglobulins are important in the prevention and treatment of various diseases in animals. Positive relationships have been reported among feeding, colostrum production, and IgG transfer (O'Doherty, Crosby

1997). Colostrum IgG concentrations are influenced by feeding nutritional components (Mazzone et al. 1999), sheep body scores at lambing, genetics, and environmental factors (Hashemi et al. 2008). IgA contents comprise 10% - 15% of blood antibodies. IgA is a class of immunoglobulin secreted onto mucosal surfaces; it prevents the intrusion of several pathogenic microorganisms into the body through mucosal membranes. Blood IgG concentrations are the highest, at 70% - 80%. IgG attaches to microbial toxins and antigens to neutralize and rapidly remove them from the body. Blood IgM comprises 10% - 15% of the body's antibodies. IgM is the first class of immunoglobulin formed in an immune response. Therefore, it plays an important role in the resistance to bacterial and protozoal parasites (Yüceer 2008). High IgM quantities may indicate an infection (Songu, Katilmiş 2012). Blood IgE content is only 0.004% (Yilmaz, Akgül 2014). IgE is transferred through the colostrum and provides protection against enterozoa (Yüceer 2008).

This study investigated changes in hematological parameters and immunoglobulin levels of sheep fed different diets. The study aimed to determine the most suitable feeding model for bolstering the immune system to contribute to successful breeding programs.

MATERIAL AND METHODS

Animals

Healthy, nonpregnant, female Norduz sheep age 1 - 2 years with live weights of 50 - 55 kg that were raised at the Agricultural Research and Implementation Center of Van Yüzüncü Yıl University constituted the animals used in this study. A total of 40 sheep comprised three groups: a control group (n = 20) and two experimental groups (n = 10 in each group). All sheep were subjected to subcutaneous administration of 0.2 mg kg^{-1} of ivermectin against internal and external parasites 2 months before the experiments. The study design complied with the ethical standards of the Provincial Directorate of Agriculture and Forestry (2020/44762815 - 1707144).

Care and feeding

For the practice of ration formulation, this study followed feeding standards developed by the US National Research Council (NRC 1985). The experiments were initiated in mid-August and lasted for 4 weeks. Randomly selected animals were divided into three groups: the control group and two experimental groups. During daylight hours, the sheep grazed primarily on low-quality pasture and lesser amounts of grass. All groups were supplied with 850 g d^{-1} of mixed grass-clover hay per head. Aside from pasture grass and mixed grass-clover hay, the control group animals were also supplied with 50 g d^{-1} of barley per head. Each animal in the experimental group (groups 1 and 2) was also supplied with barley, as follows: 65 g d^{-1} during the first week, 95 g d^{-1} during the second week, 110 g d^{-1} during the third week, and 130 g d^{-1} during the fourth week. In addition to pasture grass, mixed grass-clover hay and barley, animals from the second group were supplied with 500 g d^{-1} of concentrated feed per head throughout the experiment (4 weeks). The composition and nutrient contents of the concentrate feed are presented in Table 1. Clean and fresh water was supplied *ad libitum*.

Table 1

Composition and nutrient content of the concentrated feed provided to sheep in group 2

Ingredients	(g kg^{-1})
Barley	646.9
Soyabean meal	131.0
Wheat bran	187.6
Mineral-vitamin premix	2.5
Ground limestone	24.5
Common salt	7.5
Chemical composition (DM basis)	(%)
Dry matter	89.0
Crude Protein	17.9
Crude fiber	7.8
Ether extract	2.4
Crude ash	3.9
Calcium	1.1
Phosphorus	0.6
Metabolizable energy (Mcal kg^{-1})	3.0

Analysis of the hematological parameters

For the hematological parameters, cannulated (1.2 mm × 40 mm, 18 G) blood samples were taken from the *vena jugularis* of the sheep and placed into anticoagulant tubes with 3 mL of EDTA. The blood samples were then analyzed to determine the hematocrit (HCT) value, hemoglobin (Hb) concentration, leukocyte rate, thrombocyte (PLT) count, and mean corpuscular hemoglobin concentration (MCHC) value using a veterinary hemogram device (Veterinary MS4-s-Melet Schloesing Laboratories in France).

Analysis of biochemical parameters

For biochemical analyses, blood serum (10 mL) was taken from the *vena jugularis* and placed into no anticoagulant tubes. The blood serum samples were centrifuged at 3,000 rpm for 15 min to separate the sera. The resultant sera were preserved at -20°C until analysis began. Then, the serum IgA (Immunoglobulin A ELISA Kit SG-60915), IgE (Immunoglobulin E ELISA Kit

SG-60019), IgG (Immunoglobulin G ELISA Kit SG-60224), and IgM (Immunoglobulin M ELISA KİT SG-60112) levels were determined using the appropriate immunoglobulin kits.

Statistical analysis

The experimental results were presented as means and standard errors. One-way analysis of variance was used to hematological parameters and immunoglobulin levels. The significance of the differences between the subfactors were determined by Duncan's test according to a result of the analysis of variance. SAS statistical software (SAS 2014) was used for the statistical analyses.

RESULTS

Hematological parameters

The effects of different feeding methods on the hematological parameters of Norduz sheep are presented in Table 2. The MCHC and monocyte values

Table 2

Effects of different diets on the hematological parameters of Norduz sheep

Parameters	Control group (*n*=20) (mean ± SE)	Group 1 (*n*=10) (mean ± SE)	Group 2 (*n*=10) (mean ± SE)	*P*	Reference values#
RBC (M mm^{-3})	9.90±0.14	9.99±0.22	10.59±0.54	*ns*	9-15
HCT (%)	29.48±0.36*a*	29.60±0.76*a*	32.07±1.03*b*	*	27-45
Hb (g dL^{-1})	9.65±0.14*a*	10.68±0.19*b*	10.56±0.37*b*	**	9-15
MCV (fL)	29.37±0.26*a*	29.30±0.32*a*	31.98±1.19*b*	**	28-40
MCH (pg)	9.84±0.10*a*	10.61±0.19*b*	10.95±0.10*b*	**	8-12
MCHC (g dL^{-1})	37.85±0.30*a*	38.11±0.32*a*	40.04±0.27*b*	**	31-34
WBC (m mm^{-3})	10.91±0.30	11.59±0.26	11.11±0.29	*ns*	4-12
Leukocyte (%)	46.35±1.02	43.66±2.53	46.82±1.44	*ns*	50-75
Monocyte (%)	5.57±0.10	5.29±0.27	5.48±0.37	*ns*	0-4
Neutrophil (%)	51.00±1.13*ab*	52.67±2.14*a*	46.44±1.84*b*	*	10-50
Eosinophils (%)	5.92±0.68	6.41±0.78	4.49±0.49	*ns*	1-8
Basophil (%)	0.66±0.05*a*	0.39±0.06*b*	0.57±0.09*ab*	*	0-1
PLT (ug mL^{-1})	295.6±15.76*a*	245.3±11.20*b*	321.4±12.11*a*	*	250-750
MPV (fL)	6.51±0.05	6.56±0.08	6.52±0.07	**	-

RBC – red blood cell, HCT – hematocrit, Hb – hemoglobin, MCV – mean corpuscular volume, MCH – mean cell hemoglobin, MCHC – mean corpuscular hemoglobin concentration, WBC – white blood cell, PLT – platelet number – trombociti, MPV – mean platelet volume.

Antunovic et al. (2017)

a, *b* – different lower cases in the same line represent statistically significant differences

ns – *P*<0.05; * *P*<0.05; ** *P*<0.01.

in all groups and the neutrophil values in the control group and group 1 were higher than the reference values. The leukocyte values were lower than the reference values. The Hb and MCHC values in groups 1 and 2 were higher than these values in the control group (P<0.01). The basophil level in group 1 was higher than levels in the control group, and the PLT level was significantly lower in group 1 than in the other groups (P<0.05). The HCT level, mean corpuscular volume (MCV), and MCHC value in group 2 were higher than those in the other groups (P<0.05 for HCT and P<0.01 for other comparisons). The neutrophil level in group 2 was lower than the level in group 1 (P<0.05). The red blood cell (RBC) and white blood cell (WBC) values varied significantly among the diets. Leukocyte and neutrophil values were higher than the levels of monocytes, eosinophils, and basophils in all groups.

Biochemical parameters

The effects of different diets on the immunoglobulin levels of Norduz sheep are presented in Table 3, and the distribution of these parameters

Table 3

Effects of different diets on the immunoglobulin levels of Norduz sheep

Parametreler	Control group (n=20)	Group 1 (n=10)	Group 2 (n=10)	P
IgA (ug ml^{-1})	20.41±0.24a	21.18±0.05a	26.73±1.61b	**
IgG (ug ml^{-1})	27.22±1.18a	37.65±1.49b	37.42±1.18b	**
IgE (ng ml^{-1})	159.3±8.83a	220.2±2.45b	306.6±24.44c	**
IgM (ng ml^{-1})	15.74±0.76a	22.09±1.10b	29.99±2.15c	**

IgA – immunoglobin A, IgG – immunoglobin G, IgE – immunoglobin E, IgM – immunoglobin M
a, *b*, *c* – different lower cases in the same line represent statistically significant differences
** P<0.01

in separate groups, as box plots, is shown in Figures 1, 2, 3, and 4. The IgA levels in group 2 were significantly greater than IgA levels in other groups (P<0.01; Figure 1). The IgG levels in groups 1 and 2 were higher than those levels in the control group (P<0.01; Figure 2). Significant differences were found in the IgE and IgM levels of the all groups (P<0.01; Figures 3 and 4).

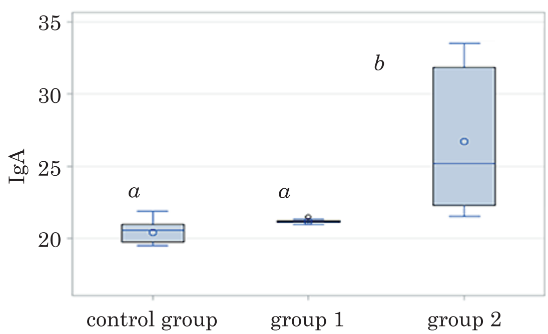

Fig. 1. Box plots for IgA levels

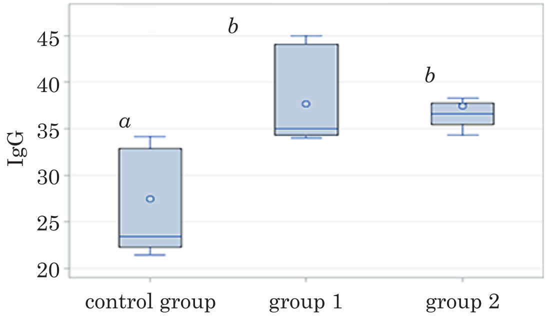

Fig. 2. Box plots for IgG levels

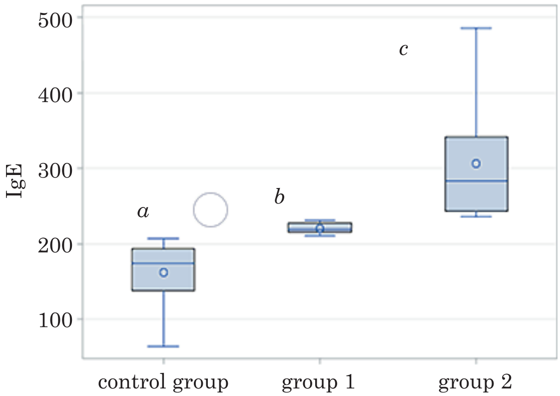

Fig. 3. Box-plots for IgE levels

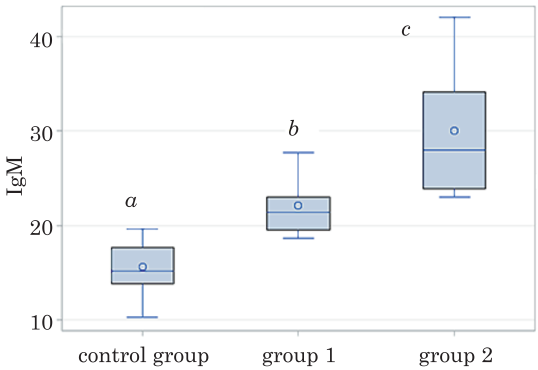

Fig. 4. Box-plots for IgM levels

DISCUSSION

Information about the basic blood parameters during the prenatal period may aid in the diagnosis of animal health and help determine necessary precautions. Diet programs that improve blood parameters and immunoglobulin levels in this period affect the affect the sheep productivity (Ahmed et al. 2018). Bozdoğan et al. (2003) investigated the effects of feeding programs on the blood parameters of Tuj sheep and reported that pasture-grazed sheep age 5 - 7 months had significantly greater Hb and erythrocyte levels compared with closed bar–fed male Tuj sheep age 2 - 3 months (P<0.05). In this study, the significantly greater HCT, Hb, MCH, and MCHC values of group 2 compared with values in the control treatment (P<0.05 for HCT and P<0.01 for other comparisons) are consistent with the findings of previous studies (Bozdoğan et al. 2003, Comba et al. 2017). This study showed that an additional supply of concentrated feed (group 2) positively affected the HCT values.

Laboratory reference values of hematological parameters are not specified for each breed, so this study used general reference values for sheep. However, identifying the reference values representing each specific breed is the best practice (Comba et al. 2017). Notably, the type of breed reportedly affects levels of some blood parameters (Arikan et al. 2001). The RBC and Hb values of all groups in this study were similar to the levels in Konya Merino sheep (10.02×10^6 mm^{-3} and 10.25 g dL^{-1}, respectively; Keçeci 1994), Karagül sheep (9.20×10^6 mm^{-3} and 10.64 g dL^{-1}, respectively; Belge et al. 1997), and Norduz sheep (Comba et al. 2017) but lower than those in the Hamdani breed (13.17×10^6 mm^{-3} and 13.53 g dL^{-1}, respectively; Eksen et al. 1992). The Hb values in this study were similar to the Hb values in Barbados Blackbelly sheep in the rainy season (10.08 g dL^{-1}) but higher than values in those sheep in the dry season (9.41 g dL^{-1}; Mohammed et al. 2014). These values were also higher than those measured in Iraqi local sheep (5.09-6.43 g dL^{-1}; Ahmed et al. 2018). Hematological parameters vary depending on breed and diet (Arikan et al. 2001, Comba et al. 2017). In a feeding study with *ad libitum* fescue grass and 300 g of concentrated feed per animal (Antunovic et al. 2011), the RBC values of Tsigai sheep were similar to those in our study; the Tsigai sheep Hb values were similar to those in the experimental groups and were higher than the control group values. Antunovic et al. (2017) fed Merinolandschaf sheep meadow grass *ad libitum* along with 600 g of concentrated feed per animal and reported lower RBC and Hb values than those observed in our study.

The results of this study revealed that the HCT values of all the groups were lower than those of Hamdani sheep (41.41%; Eksen et al. 1992) and Tuj sheep (34.27%; Çelebi, Uzun 2000) but were higher than levels in Norduz sheep (26.40%) reported by Comba et al. (2017). Group 2 HCT values were similar to those observed in Konya Merino sheep (31.00%), as reported by

Keçeci (1994), and in Morkaraman sheep (32.50%), as reported by Çelebi, Uzun (2000); Group 2 HCT values were higher than levels measured in Karagül sheep (28.30%; Comba et al. 2017).

The MCHC value is important for the diagnosis of anemia and is a beneficial index to identify the RBC production capacity of the bone marrow (Njidda et al. 2014). The MCHC values of this study were higher than the reference values reported by Antunovic et al. (2017). MCV and MCHC values reported by Yilmaz, Emre (1981) for Norduz sheep fed under extensive conditions were lower than those of all groups in this study. The MCV and MCHC values in Merinolandschaf sheep fed meadow grass *ad libitum* plus 600 g of concentrated feed per animal were higher than those values of all groups in this study (Antunovic et al. 2017). The MCHC (27.11-29.55 g dL^{-1}), MCV (0.64-1.15 fL), and MCH (0.227-0.277 pg) values in Iraqi local sheep fed a diet similar to that in the Merinolandschaf sheep were lower than the levels in all the groups in this study (Ahmed et al. 2018).

WBCs are blood cells that participate in the defense system (Comba et al. 2017). WBC increases may be caused by colostrum or may emerge as an immune system response to microbes to which pasture-grazed animals are exposed, especially in extensive rearing systems (Wada et al. 2014). The WBC values of Hamdani sheep (Eksen et al. 1992) and Konya Merino sheep (Keçeci 1994), at 5.55×10^3 mm^{-3} and 5.27×10^3 mm^{-3}, respectively, were lower than the values of all the groups in this study.

Increases in leukocytes, monocytes and neutrophils could be a response to inflammation or stress (Poitout-Belissent, McCartney 2010, Wada et al. 2014). The slight increases in the monocyte (in all groups) and neutrophil (in the control group and group 1) ratios observed in this study can be considered to be a response to various inflammatory processes. Increased eosinophils and basophils are rarely seen in toxicological studies when the test material is not a hematopoietic growth factor or a cytokine (Poitout-Belissent, McCartney 2010). In this study, these values were found to be compatible with the reference values.

In this study, in addition to pasture grazing, feeding with mixed grass-clover hay and extra barley (group 1) was sufficient to increase Hb and MCH parameters. It was determined that giving concentrated feed in addition to this feeding (group 2) significantly increased HCT, MCV, and MCHC parameters compared with other groups.

Newborn lambs have limited energy reserves, and they have an urgent need for colostrum to sustain homeothermy and survive. Aside from energy, colostrum also provides immunoglobulins, which provide passive systemic immunity (Nowak, Poindron 2006). The survival rate of lambs in the neonatal period largely depends on the age of the ewes, the lamb birth weight, the type of birth, the lamb gender, and dietary conditions (Hatcher et al. 2009). Meeting the needs of ewes in the prenatal period ensures sufficient and quality colostrum supply (Banchero et al. 2004). In ruminant animals, transmission of maternal immunity to the fetus is blocked by the placenta (Kozat 2019). Feeding plays a great role in the antibody production of ewes, so it is considered an important immunoglobulin factor that influences the level of passive immune transfer to lambs (Kozat 2018, Eğlenti et al. 2020). In this study, the immunoglobulin levels in the experimental groups (groups 1 and 2) fed different diets were significantly higher than levels in the control group (Table 3). Specifically, the IgA (Figure 1), IgE (Figure 3), and IgM (Figure 4) levels in animals in group 2 (fed with enriched, concentrated feed) were significantly higher compared with the other groups ($P<0.01$). The IgG level in group 2 was similar to that in group 1 (Figure 2). The IgA levels in sheep in the control group, fed the poorest diet, were similar to levels in group 1. However, other immunoglobulin levels in the control group were lower than those in groups 1 and 2 ($P<0.01$). In addition, significant increases ($P<0.01$) were observed in the IgE levels (Figure 3) and IgM levels (Figure 4) as diet quality improved (Table 3).

CONCLUSIONS

This study revealed that improved dietary quality causes significant increases in hematological parameters and immunoglobulin levels. In addition to pasture grazing, feeding with mixed grass-clover hay and extra barley increased the Hb levels, MCH parameters, and IgG levels. Adding concentrated feed to the hay and barley feeding resulted in significant increases in HCT, MCV, and MCHC parameters and in IgA, IgE, and IgM levels. Improvements in these values will reduce the risk of disease in animals and naturally increase their productivity.

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