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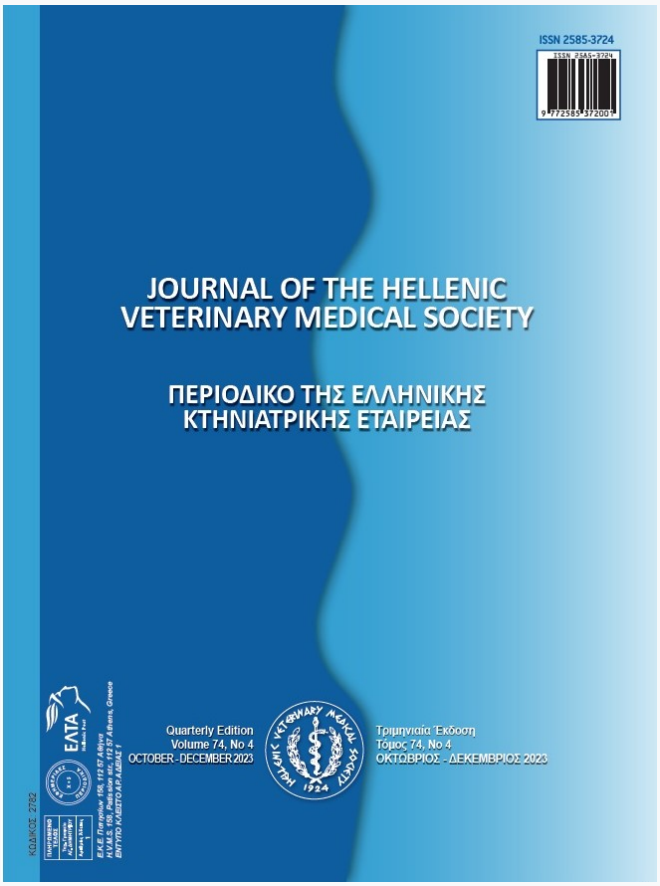

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Effects of whey-enriched drinking water on fattening merino lamb growth, hemogram, inflammation, oxidant and antioxidant parameters

E. Danyer[1], T. Bilal[2], A. Altıner[3], H. Eseceli[4]

[1]*Veterinary Control Research Institute, Ankara, Türkiye*

[2]*Department of Animal Nutrition and Nutritional Diseases, Veterinary Faculty of Istanbul University- Cerrahpasa Istanbul, Türkiye*

[3]*Department of Biochemistry, Veterinary Faculty of Istanbul University- Cerrahpasa Istanbul, Türkiye*

[4]*Department of Nutrition and Dietetics, Health Sciences Faculty of Bandirma Onyedi Eylül University Balikesir, Türkiye*

ABSTRACT: The goal of this study was to find out how drinking whey-enriched *ad-libitum* water affected oxidative stress and inflammation in 24 Merino lambs that were putting on weight. To do this, lambs were randomly split into two groups and given either*ad libitum* freshwater (control) or whey-enriched water (whey), clover as roughage, and lamb grower concentrate feed as their daily ration. Blood samples were collected before the trial (T1), on the 15th (T2),), and 30th (T3) days, and hemogram; serum antioxidant enzymes (Superoxide dismutase, SOD; Glutathione peroxidase, GSH-Px); inflammation response (C-Reactive Protein, CRP), complement activation; chemokine markers (Complement Component 4, C4), lymphocyte activation factor (IL-1), systemic inflammation cytokines. The rate of hematocrit was found to be higher in the experimental group in T3 (35. 55 ± 10. 54) compared to the control group (44. 50 ± 2. 58). T2 and T3 showed higher platelet amounts in the whey group than the control group (p<0. 05). In conclusion, with the use of whey-enriched drinking water, no significant change was observed in the amounts of TNF-α, SOD, MDA, IL-1β, C4, CRP and live weight gain under these study conditions between the two groups.

***Keywords*:** Blood biochemical parameters;inflammation effect;feed additive;merino lambs;oxidation efficiency;whey powder

Corresponding Author:
E. Danyer, Veterinary Control Central Research Institute, Ahmet ŞefikKolaylı Cad. No 21 06020 Esertepe, Keçiören, Ankara, Turkey
E-mail address: erdemdanyer@gmail. com

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INTRODUCTION

Whey is a broad name for the clear liquid fraction-of milk that remains after the cheese-making processes like coagulation and curd removal (Hoffman and Falvo, 2004). In addition to branched-chain amino acids, vitamins, and minerals, whey proteins contain a high level of essential amino acids (Hoffman and Falvo, 2004). Whey proteins are separated from this liquid by various methods. While 80% of the whey obtained from cow milk, which contains approximately 3. 5% total protein, consists of casein, the remaining 20% of it consists of whey proteins (Hoffman and Falvo, 2004; Hulmi et al., 2010)considering the variety of proteins that are available much less is known concerning the benefits of consuming one protein versus another. The purpose of this paper is to identify and analyze key factors in order to make responsible recommendations to both the general and athletic populations. Evaluation of a protein is fundamental in determining its appropriateness in the human diet. Proteins that are of inferior content and digestibility are important to recognize and restrict or limit in the diet. Similarly, such knowledge will provide an ability to identify proteins that provide the greatest benefit and should be consumed. The various techniques utilized to rate protein will be discussed. Traditionally, sources of dietary protein are seen as either being of animal or vegetable origin. Animal sources provide a complete source of protein (i. e. containing all essential amino acids. In a study, the differences within groups, however, were determined to be insignificant in the control group, where the lack of whey as an energetic feed ingredient resulted in a decreased quantity of intramuscular and sternal fat (Dayani et al., 2011). The major whey proteins of cow milk are 55% β-immunoglobulin, 20% α-lactalbumin, and 7% albumin (El-Agamy, 2007). Leucine is the main amino acid in whey and whey is rich in the amino acid cysteine (Hoffman and Falvo, 2004; Pal and Radavelli-Bagatini, 2013) considering the variety of proteins that are available much less is known concerning the benefits of consuming one protein versus another. The purpose of this paper is to identify and analyze key factors in order to make responsible recommendations to both the general and athletic populations. Evaluation of a protein is fundamental in determining its appropriateness in the human diet. Proteins that are of inferior content and digestibility are important to recognize and restrict or limit in the diet. Similarly, such knowledge will provide an ability to identify proteins that provide the greatest benefit and should be consumed. The various techniques utilized to rate protein will be discussed. Traditionally, sources of dietary protein are seen as either being of animal or vegetable origin. Animal sources provide a complete source of protein (i. e. containing all essential amino acids. Cysteine increases glutathione levels and enhances antioxidant capacity (Margaritelis et al., 2020). It was reported that lactoferrin in whey had a regulating effect on cytokine release, and this function might aid in the prevention of tissue-damaging inflammation (Håversen et al., 2002; Mattsby-Baltzer et al., 1996).

Physiological and psychological stresses can cause the release of proinflammatory cytokines such as IL-1, IL-6, and TNF-αare thought to be major early inducers of organ damage. In humans, IL-1 is a proinflammatory cytokine with a razor-thin boundary between therapeutic benefit and severe damage (Dinarello, 1996). C-Reactive Protein (CRP) is an acute-phase protein; its levels correlate well with the intensity of inflammation, and its increase may indicate postoperative complications (Gabay and Kushner, 1999). At the same time, CRP has several cardiovascular effects (clotting, the production of oxygen radicals, an increase in the expression of adhesion molecules, plasminogen activator inhibitor-1, and plaque destabilization) that may lead to cardiovascular disease (Prasad, 2006). C4 is part of the complement system and has a modulator role during inflammation (Danyer et al., 2021). GSH-Px and SOD can decrease oxidative production by preventing lipid peroxidation. GSH-Px can facilitate the reaction between hydrogen peroxide (H_2O_2) and reduced glutathione to generate water and oxidized glutathione. SOD is a key component of the antioxidant system, which eliminates superoxide and protects cells from injury and MDA is a naturally occurring mutagenic and carcinogenic (Wang et al., 2011) naturally occurring product of lipid peroxidation and prostaglandin biosynthesis (Marnett, 1999). Complement is a very important component of innate immunity and uses both foreign microbial pattern recognition and antigen-specific activation (Mcgreal et al., 2012).

Whey has wide usage area in farm animal nutrition. Whey protein concentrate is one of the most commonly used sources of animal protein in the diet of piglets (Zhao et al., 2014). Since whey is quite cheap, it can also be a source of nutrients for lambs and calves in the growing period (Kushibiki et al., 2001). It is known that the use of whey as a feed ingredient for lactating goats provides advantages in reducing feed costs and preventing negative environmental effects (Rapetti et al., 1995). Many studies have been conducted on whey

that subjecting ruminants. In a study, the addition of whey caused the fermentation of potato mixed silage and it was concluded that better feed conversion ratios would be obtained with whey-potato mixed silage compared to other diets ($p< 0.05$) (Nkosi and Meeske, 2010). It was determined that the use of whey increased the cow's live weight gain (Galloway et al., 1993). In another study conducted parallel with this study at the same time, it was determined that the use of whey in lambs increased the final loin eye depth by 4. 7 times (Eseceli et al., 2021). Also, it is found that whey additives had an association with the intraluminal liquid flow rate and the amount of uric acid excreted by urine increased in lambs (Susmel et al., 1995). It was found that colostrum whey had a stimulating effect on serum complement activity in newborn calves (Rokka et al., 2001). In a study conducted on Alpine and Saanen goats, it was determined that the use of whey with 6. 5% total solid content increased the milk yield and fat ratio, curd firmness, and coagulation time (Rapetti et al., 1995). Also, ruminants can handle up to 30% of their total dry matter intake as whey without having negative side effects (Schingoethe, 1976). The use of whey has been discussed in many studies in the nutrition of ruminants for more than fifty years (DePeters et al., 1986; Rapetti et al., 1995; Schingoethe, 1976; Susmel et al., 1995; Thivend, 1978; ZoBell et al., 2005) however, there are not many studies on the inflammation, oxidant, and antioxidant parameters in lambs.

It is known that oxidative stress contributes to numerous pathological conditions, including ageing and muscle fatigue. So, fattening performance has a close association with oxidative parameters. Whey effects need to be evaluated since these parameters significantly affect body weight gain and health status. In animal-sourced production, producers should consider animal welfare. Another reason for the importance of the animal's welfare is the association with animal performance. For the reasons listed, it is important to observe and evaluate inflammation and stress parameters. In previous studies, the focus topics were mainly concentrated on the performance parameters. In this study, besides live weight performance, animal welfare was also wanted to be observed. Another contribution of this study to the literature will be to evaluate the possibility of using whey by dissolving it in the water.

The objective of this study is to evaluate the effects of whey used with drinking water on the selected hemogram, serum iron, lymphocyte activation factor (IL-1β), systemic inflammatory cytokine (TNF-α), inflammation response parameter (CRP), complement activation, chemokine marker (C4), serum antioxidant enzymes (SOD and GSH-Px), lipid peroxidation marker (MDA), andlive weight alteration performance in weaned three-month-old male lambs.

MATERIALS AND METHODS

Animals, study design and power analyses

In this study, 24 weaned, three months old Merino lambs (23. 44 ± 0. 62 kg) were divided randomly into two equal groups, each containing 12 lambs. The power analysis of the study was performed with the G Power 3. 1. 9. 7 program for repeated measures in ANOVA, and when the effect size was taken as f:0. 28 with α error probability: 0. 05.

Experimentalration

While the control group had *ad libitum* freshwater, the whey group had *ad libitum* whey powder (Astosan, Gönen, Balıkesir) added to drinking water. From mid-March to early-May 2019 in Gönen/Balıkesir (40º 6′ 28. 7058′′N, 27º 38′ 12. 0192′′E), 6. 56 g of whey powder was dissolved in a small container before being put into the whey group water tank and completing the 100 L final capacity for enhancing liquid whey (Eseceli et al., 2021). According to the manufacturer's recommendations, the daily concentration was determined to be 65 ppm/day for 12 lambs. Whey powder was transformed into liquid whey and added to drinking water due to its simplicity of transportation and storage and minimal risk of degradation. According to Thivend (1978), at least one week should be established for the adaptation of rumen microorganisms before the whey is used in ruminants. Therefore, the experiment was performed for 30 days after 15 days of adaptation. The two groups were fed the same ration, and the drinking water that they consumed only *ad libitum* varied as to whether it was whey-enriched (Whey group) or not supplemented (Control group). Body weights of lambs were recorded on days 0 (initial, T1), 15 (mid, T2), and30 (final, T3) of the study. All recordings were done on the same day. The feed was sampled at the beginning of the study, and all animals received the same batch during the study. Feed analyses were undertaken according to the Weende analysis system. Detailed information can be found on feed and whey analyses in (Eseceli et al., 2021).

Sample Collection Procedure

On day 0 (T1) during which the study started, and on days 15 (T2) and 30 (T3) of the experiment, before

the first feeding, blood samples were collected from the jugular vein with a 21-gauge vacuum tube cannula into a self-vacuum tube with K_3EDTA (Vakutest® Kima) for hemogram analyses and 8 ml gel blood tubes for serum biochemical analyses. After blood collection, the samples were brought to the laboratory in a cold chain and centrifuged at 22°C for 10 minutes at 2000 g to obtain serum. Serum samples were stored at -80°C until analysis and were slowly thawed at 4°C before analysis.

Laboratory analyses

Hemogram analyses of red blood cells (RBCs), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), and CRP were done with the reference materials recommended by the manufacturer and calibrated with the calibration liquid before each use. Automatic blood count equipment (Mindray® BC2800 Vet) was used to look at the blood samples within a maximum of 24 hours. The enzyme-linked immunosorbent assay (ELISA) method was used to determine the biochemical parameters because it was fast, easy to use, accurate, and precise. ELISA kit brands and catalog numbers are listed: GSH-Px (MyBioSource, MBS739813), C4 (Sunredbio, 201-07-0386), IL-1β (Sunredbio, 201-07-0010), MDA (Sunredbio, 201-07-0086), SOD (Sunredbio, 201-07-1566), and TNF-α (Sunredbio, 201-07-0060). The ELISA method was performed according to the kit instructions. ELISA kits and hemogram analyzer maintenance and validation procedures were performed according to the manufacturer's recommendations. Materials for validation and internal quality control solutions were provided by the ELISA kit and hemogram analyzer manufacturers. Serum iron analyses were carried out with an inductively coupled plasma mass spectrometer (ICP-MS) (Agilent 7700x, Agilent Technologies®) after pretreatment in the microwave (Berghof Instruments).

For quality control of the analyses, all samples were tested in duplicate, and differences of 0. 05% were accepted between duplicate analyses. The intra-assay coefficient of variation (CV) and the inter-assay CV were determined by analyzing six samples on three different occasions.

Statistical analyses

Before starting the randomization of the lambs, the Grubbs test was performed to see if there was a significant difference in the live weight. The normal distribution of the results was controlled using the Shapiro-Wilk test, and the homogeneous distribution of variances was controlled by Levene's test. The general linear model of repeated measurements was used in the comparison of repeated measurements. The results were examined in terms of diet and time effects according to the model below.

$$Y_{ij}=\mu+\alpha_i+\beta_j+(\alpha\beta)_{ij}+\varepsilon_{ij}$$

Where;

Y_{ij} is the dependent variable, μis the overall mean, α_i is the systemic effect of whey enriched or normal drinking water, β_j is the systemic time effect (T1, T2, T3), $(\alpha\beta)_{ij}$ and the ε_{ij} is the random error.

The results were reported as group means and standard error means. The Tukey Multiple Range Test was applied to look at differences after the fact, and the dependent samples t-test was conducted to look at differences between the two times. Friedman and Mann-Whitney U tests were used to evaluate the non-normally distributed parameters (HCT, MCV, MCH, MCHC, and GSH-Px). A binomial logistic regression was used to identify independent predictors of outcomes for the multivariate analyses. For this purpose, lambs' 120th day weight was calculated as the cutoff value. The independent variable is the drinking of whey enriched water or not, the body mean weight of first, middle, and last days, and age (in days); the dependent variable is the weight of the last body, as well as whether it is over or under 37. 2 kg. Backward elimination was used to determine the initial model. Hosmer-Lemeshow goodness-of-fit statistics were used to assess model fit. The Jamovi V. 1. 8. 1 program was used in the statistical evaluation. The limit of statistical significance was considered as $p<0.05$ for all analyses. The data that support the findings of this study are available from the corresponding author upon reasonable request.

RESULTS

Study ration, live weight gains results

Table-1 (concentrate feed ingredients) and Table-2 (chemical make-up of the concentrate, roughage, and whey used in the study) show the concentrate and roughage nutrient content of the experiment. Prior to randomizing the lambs, initial live weight differences were checked to control the significant outliers by the Grubbs test, and it was concluded there were no outliers in the live weight of all lambs. Live weight measurements have been shown in Table-3 and displayed in Figure-1.

Table 1. Ingredients of concentrate of the study ration (Eseceli et al., 2021).

Item	Amount (g/kg)	Item	Amount (g/kg)
Wheat bran	251	Rice bran	35. 5
Corn grain	170	Limestone	31
Corn germ meal	127. 3	Ammonium chloride	6
Barley	100	Molasses	5
Sunflower meal	96. 4	Provin	5
Bonkalite	80	Salt	3
Linseed meal	50	Vitamin Mineral Premix[2]	1. 5
DDGS[1]	38. 3		

[1]Distiller's dried grains with soluble.
[2]150 mg $ZnSO_4$. $7H_2O$, 80 mg $MnSO_4$. H_2O, 200 mg MgO, 5 mg $CuSO_4$. $7H_2O$, 1 mg KIO_3, 5000 IU vitamin A, 1000 IU vitamin D, and 20 IU vitamin E.

Table 2. Dry-matter nutrient levels and calculated energy content of the study ration (as dry matter) (Eseceli et al., 2021).

Item	Concentrate	Wheat straw	Whey powder
Dry matter (g kg^{-1})	875. 8	927	974. 1
Crude protein (g kg^{-1})	225. 2	48. 0	174. 3
Crude Ash (g kg^{-1})	95. 1	76. 1	85. 3
Crude fat (g kg^{-1})	75. 9	16	19
Crude fiber (g kg^{-1})	83. 3	323. 6	-
NDF (g kg^{-1})	227. 3	730. 5	-
ADF (g kg^{-1})	105. 1	494. 4	-
ADL (g kg^{-1})	40. 4	88	-
NFC (g kg^{-1})	376. 3	129. 4	721. 4
DE (MJ kg^{-1})	12. 76	10. 20	12. 79

Abbreviations: NDF: Neutral Detergent Fiber; ADF: Acid Detergent Fiber; ADL: Acid Detergent Lignin; NFC: Non-Fiber Carbohydrate.

Table 3. Live weight (kg)measurements at 90th (T1), 105th (T2)and 120th (T3) days (Mean ± SD).

Parameter	Group	Mean ± SD			SEM	*p-value*	
		T1	T2	T3		Group	Time
Live weight (kg)	Control	23. 51 ± 3. 98[a]	29. 76 ± 5. 23[ab]	36. 27 ± 5. 28[b]	1. 11	0. 8	0. 001*
	Whey	23. 37 ± 1. 8[a]	30. 10 ± 2. 74[ab]	37. 22 ± 3. 69[b]			

*: *p*-value<0. 05, Values in the same row, appearing in pairs differ at a, b, c.
Abbreviations: SD: standard deviation; SEM: standard error of the mean.

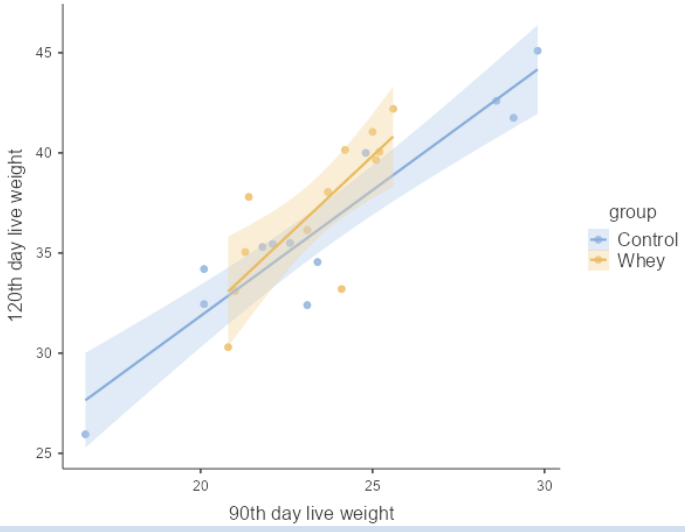

Figure1. Scatter plot of the 90th day to120th day live weight (kg) measurements of the individuals with ± 2 standard deviation confidence intervals.

As expected, all animals' live weights were in increasing trend in every measurement time. However, whether they drink water that has whey added to it or not has no effect on their live weight. In the binominal logistic regression model with -2 Log-likelihood, andwere found to be 13. 25, 0. 56, and 0. 75, respectively. The Chi-square and significance of the Hosmer-Lemeshow test were 6. 76 and 0. 56, respectively, for Hosmer-Lemeshow test. Binominal logistic regression analysis findings showed that a 90thlive weight has a 3. 46-fold effect (odds ratio) to gain one more kg on the 120th day (p=0. 02) (Table 4).

Power of the study and quality control results of the analyses

The power of our study (1-β error probability) for three repeated measurements was calculated as 0. 83.

The intra-assay coefficient of variation (CV) was determined by duplicate analyses of certain samples and was below 3. 9%; the inter-assay CV (6. 8%) was determined by analyzing six samples on three different occasions.

Hemogram results

It was determined that the erythrocyte and hemoglobin values were within the limits at the three measurement times and that there was no difference between the groups (Table-5). Although it was observed that the hematocrit value of the two groups was slightly outside the limits at the beginning of the study and remained below the lower limit in the second measurement, it was observed that the percentage of hematocrit in the whey group (43. 18 ± 9. 85%) was higher in comparison to the control group (35. 55 ±10. 54%), although it was within the reference values in T3 (p<0. 05). It was determined that the MCV value was higher compared to the control group and outside

Table 4. Logistic regression model for 90th day live weight (kg) and group (drinking water type) differences.

Parameters	β	S. E.	Wald	f	p-value	Exp (β)	95%C. I. for p- value	
							Lower	Upper
Constant	-30. 61	12. 45	6. 05	1	0. 1	0. 01*		
90. day live weight	1. 24	0. 52	5. 66	1	0. 02*	3. 47	1. 24	9. 65
Drinking water type	2. 05	1. 52	1. 81	1	0. 8	7. 75	0. 39	152. 45

Abbreviations: SE:Standard error. C. I:Confidence interval. *: p-value<0. 05.

Table 5. Red blood cell (RBCs), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), and serum iron (Fe) results were compared with reference values (Mean ± SD).

Parameter	Group	Mean ± SD			SEM	p-value	
		T1	T2	T3		Group	Time
RBCs (10^{12}/L) [9-15]	Control	10. 23 ± 0. 83	10. 28 ± 0. 85	10. 20 ± 0. 96	0. 15	0. 20	0. 51
	Whey	10. 74 ± 1. 01	10. 90 ± 0. 81	10. 91 ± 1. 07	0. 16		
HGB (×10 g/L) [9-15]	Control	12. 68 ± 0. 94	11. 89 ± 0. 95	11. 71 ± 1. 21	0. 24	0. 97	0. 33
	Whey	10. 85 ± 2. 15	12. 66 ± 0. 90	12. 64 ± 1. 14	0. 23		
HCT (%) [27-45]	Control	46. 31 ± 3. 64^{a}	25. 60 ± 2. 18^{b}	35. 55 ± 10. 54^{aB}	1. 08	0. 001*	0. 01*
	Whey	46. 30 ± 5. 35^{a}	25. 23 ± 2. 18^{b}	43. 18 ± 9. 85^{aA}	1. 12		
MCV (fL) [28-40]	Control	45. 38 ± 2. 35^{a}	24. 97 ± 1. 52^{c}	34. 55 ± 8. 06^{bB}	0. 80	0. 03*	0. 01*
	Whey	44. 25 ± 3. 43^{a}	23. 15 ± 1. 37^{b}	44. 50 ± 2. 58^{aA}	0. 82		
MCH (pg) [8-12]	Control	12. 37 ± 0. 77^{A}	11. 51 ± 0. 50	11. 43 ± 0. 62	0. 17	0. 002*	0. 46
	Whey	10. 40 ± 1. 64^{B}	11. 57 ± 0. 46	11. 54 ± 0. 64	0. 16		
MCHC (×10 g/L) [31-34]	Control	27. 38 ± 1. 52^{cA}	46. 45 ± 1. 31^{aB}	34. 70 ± 7. 10^{bA}	0. 74	0. 006*	0. 001*
	Whey	23. 33 ± 2. 88^{bB}	50. 30 ± 2. 16^{aA}	26. 03 ± 0. 81^{bB}	0. 71		
PLT (10^{9}/L) [280-650]	Control	681. 00 ± 102. 10^{a}	458. 75 ± 115. 90^{bB}	432. 58 ± 177. 06^{bB}	34. 55	0. 002*	0. 001*
	Whey	770. 91 ± 359. 56^{a}	584. 50 ± 164. 03^{cA}	667. 41 ± 164. 88^{bA}	33. 52		
Iron (Fe) µmol/L [11. 46-40. 11]	Control	93. 63 ± 35. 61	90. 66 ± 73. 17	46. 54 ± 10. 44	11. 71	0. 94	0. 22
	Whey	65. 24 ± 26. 81	92. 57 ± 29. 06	75. 90 ± 38. 77	7. 58		

*: p-value <0. 05. Values in the same row, appearing in pairs differ at a, b; values in the same column, appearing in pairs differ at A, B. Abbreviations: SD:Standard deviation; SEM:Standard error of the mean. Iron reference intervals obtained fromhttps://www. vet. cornell. edu/animal-health-diagnostic-center/laboratories/clinical-pathology/reference-intervals/chemistry

the reference limits at T3. MCH and MCHC levels vary according to other hemogram parameters. Platelets dropped in both groups based on measurement times, and they were found to be significantly greater in T3 in the whey group compared to the control group in T3 (p<0. 05). Both groups' serum iron levels were unaffected by enriched whey drinking water.

Biochemical results

Although there was no difference between the groups in aspects of the inflammation response parameter (CRP), complement activation and chemokine marker (C4), lymphocyte activation factor IL-11, systemic inflammation cytokine (TNF-α, MDA, GSH-Px, and SOD) concentrations (Tables 6 and 7), the GSH-Px concentration increased slightly within the two groups at each measurement time. Between T1 and T3, the rising trend in GSH-Px concentration was around 7 U/L in the whey group and 20 U/L in the control group, as shown in Table-7.

DISCUSSION

It has been reported that whey can be used as a source of energy and nitrogen for ruminants (Thivend, 1978). Ruminants may consume up to 30% of their dry matter as liquid whey without experiencing performance issues, but pigs may have diarrhea if they consume more than 20% of their dry matter as liquid whey (Schingoethe, 1976). There have been studies on the use of whey in ruminants for many years. However, there are not enough studies on the blood parameters discussed in this study.

It was observed that the HCT value was higher in the whey group at the T3 measurement. It has previously been reported that using liquid whey may cause excessive urination (Schingoethe, 1976; Susmel et al., 1995). Although the amount of urination was not recorded in this study, there was no difference in HCT levels between the two groups at T1; however, the fact that the percentage of HCT was found to be significantly greater in the whey group at T3 suggested that the whey group may have more water loss compared to the other group. Repeating the experiment in hot climate conditions will reveal this situation more clearly. The reason for this higher value was considered to be because they drank water containing more dissolved substances. In a study conducted during 30 minutes of training in basketball players, RBC, HGB,

Table 6. Inflammation response parameter [C-Reactive Protein (CRP)], Complement Activation and Chemokine Marker [Complement Component 4 (C4)], Lymphocyte Activation Factor [Interleukin 1 Beta (IL- 1 β)], Systemic Inflammation Cytokine [Tumor Necrosis Factor-α (TNF-α)], [Malondialdehyde (MDA)] concentrations of the lambs (Mean ± SD).

Parameter	Group	Mean ± SD			SEM	*p*-value	
		T1	T2	T3		Group	Time
CRP (mg/dL)	Control	0. 34 ± 0. 06	0. 35 ± 0. 11	0. 32 ± 0. 06	0. 16	0. 35	0. 90
	Whey	0. 37 ± 0. 14	0. 34 ± 0. 07	0. 31 ± 0. 05	0. 16		
C4 (ng/mL)	Control	23. 35 ± 10. 42	24. 31 ± 4. 558	32. 54 ± 18. 16	2. 03	0. 33	0. 12
	Whey	19. 37 ± 4. 22	26. 95 ± 18. 18	25. 03 ± 7. 45	2. 06		
IL-1β (ng/mL)	Control	5. 04 ± 1. 98	4. 65 ± 1. 29	6. 64 ± 3. 47	0. 46	0. 80	0. 32
	Whey	5. 46 ± 4. 27	4. 91 ± 2. 94	5. 38 ± 1. 16	0. 47		
MDA (nmol/L)	Control	1. 55 ± 0. 65	1. 57 ± 0. 34	2. 59 ± 1. 36	0. 21	0. 74	0. 34
	Whey	1. 81 ± 2. 05	1. 79 ± 1. 68	1. 74 ± 0. 35	0. 21		
TNF-α (ng/L)	Control	13. 53 ± 6. 02	13. 73 ± 3. 18	19. 28 ± 10. 34	1. 63	0. 89	0. 65
	Whey	15. 51 ± 14. 82	15. 28 ± 13. 29	14. 40 ± 2. 93	1. 63		

Abbreviations: SD:Standard deviation; SEM:Standard error of the mean.

Table 7. Glutathione Peroxidase (GSH-Px) and Superoxide Dismutase (SOD) concentration results (Mean± SD).

Parameter	Group	Mean ± SD			SEM	*p*-value	
		T1	T2	T3		Group	Time
GSH-Px (U/L)	Control	137. 50 ± 41. 71^{c}	147. 80 ± 53. 34^{ab}	156. 83 ± 57. 63^{a}	6. 86	0. 42	0. 03*
	Whey	130. 54 ± 25. 18^{b}	119. 83 ± 19. 05^{c}	137. 27 ± 30. 64^{a}	6. 85		
SOD (ng/mL)	Control	3. 58 ± 1. 93	3. 33 ± 0. 77	4. 96 ± 2. 73	0. 36	0. 88	0. 44
	Whey	3. 96 ± 3. 17	3. 81 ± 2. 28	3. 77 ± 0. 91	0. 36		

*: p-value<0. 05. Values in the same row, appearing in pairs differ at a, b, c. Abbreviations:SD: Standard deviation; SEM:Standard error of the mean.

and HCT values were found to be higher in the whey group compared to the control group, in parallel with our study (Ronghui, 2016).

The use of whey led to an increase in MCV. According to MCVdata, it was found to be compatible with the study of Hassan et al., (2016)although it was outside the previously stated reference values (Byers and Kramer, 2011). To explain the hemogram changes and anemia markers, the blood serum iron level was determined. There was no significant difference between the two groups, but the whey group means serum iron concentration was found above reference levels. Iron in foods is ionized by stomach acids, absorbed by the intestines, and then circulated in the blood (Bilal, 2004). Lactoferrin is a well-known bioactive protein in milk that helps to make iron more bioavailable for absorption in the gut (Ribadeau-Dumas and Grappin, 1989). Iron-whey protein microspheres may protect gastrointestinal epithelial cells and increase iron absorption compared to $FeSO_4$ in fasting adults (Wang et al., 2018)resulting in the widespread use of high-dose oral iron, which is poorly tolerated. Methods: We evaluated novel iron-denatured whey protein (Iron-WP. Spray-dried whey protein concentrate-iron complex supplementation increased iron digestion and metabolic utilization while maintaining iron homeostasis and hepatic iron reserves in weaning and anemic rats (Gandhi et al., 2019). In addition, evaluation of the MCV values revealed that the MCV value dropped in the control group at T3, but it remained the same in the whey group. In the control group, serum iron content was shown to decrease. When the MCV and serum iron findings are compared, it is assumed that the control group absorbs less iron, and as a result, the MCV value lowers. According to the explanation given, the serum iron level in the whey group is higher.

MCHC value is lower in the whey group compared to reference values. MCHC drop could occur in the presence of reticulositis (Bilal, 2004). It appears that both groups are not anemic, but according to the literature, one group could have reticulositis. Reticulosit presence was not evaluated in these study conditions. There is a possibility that reticulosit evaluation could be a useful parameter in future studies.

The whey group's PLT content increased significantly at T2 and T3. Although several peptides derived from k-casein and lactoferrin are antithrombotic and platelet aggregation inhibitors (Rutherfurd and Gill, 2000), in one previous study, rats were given two dosages of whey protein concentrate (WPC-80) (0. 3 or 0. 5 g/kg) for 7, 14, or 21 days to see how it affected the development of thrombosis, and it was found that there were minor changes in andour findings are consistent with those (Tokajuk et al., 2019).

The GSH-Px concentration increased over time, which is a situation that can be encountered during a growing period parallel to ageing. Although there was no statistically significant difference between the groups, the control group demonstrated increased GSH-Px consistent with an upregulation of GSH-Px to protect against oxidative stress. Parallel to our findings, previously it was suggested that whey protein improved antioxidant capacity against acute oxidative stress through multiple potential mechanisms, including glutathione production. The glutathione-promoting activity of whey protein may contribute to a broader biological effect of a protective nature in regard to general detoxification of environmental agents (Xu et al., 2011). Whey protein decreased glutathione activity to levels substantially higher than those of the control group, and these effects persisted in the presence of H_2O_2.

Whey protein could be used as an alternative source of antioxidants to prevent damage caused by reactive oxygen species. The concentrations of C4, IL-1, MDA, TNF-α, and SOD were also found to be higher in the control group. Antioxidant enzymes neutralize the harmful effects of radicals formed as a result of increased oxidative stress. Increased levels or activation of these endogenous enzymes have been shown to protect cells against oxidative damage. The findings suggest that whey protein improves antioxidant capacity and that this protein may serve as an alternative source of antioxidants for the prevention of oxidative damage. On the other hand, this study was designed without the addition of any additional stressors. The effect of whey protein on stress parameters can be revealed more clearly in future studies by using stress factors such as transport stress.

The live weight on the 90th day had a substantial effect on whether or not the lambs' live weight on the 120th day was greater than or less than the cutoff value of 37. 2 kg. According to the findings of this study, whey protein has no significant impact as a fattening supplement. In a previous study, the addition of 10% whey and 10% molasses to corn silage decreased pH, ash, neutral detergent fiber, and acid detergent fiber, while increasing dry matter, crude protein, organic matter, and flieg point, but had no effect on feed

intake, body weight, or feed conversion ratio (Fallah, 2019). Which accords with the findings of this study. In contrast with our study, the addition of urea and whey to wheat straw improved the feed conversion ratio and increased the body weight of fattening lambs (Dayani et al., 2011). There is a close relationship between feed efficiency during the fattening period, gluconeogenesis, the tricarboxylic acid cycle, and protein synthesis (Giráldez et al., 2021). According to this study's results, whey-enriched drinking water did not modify any of these pathways. This could be due to the small quantity of whey used. In future research, it is suggested that the amount of whey be increased proportionally.

This study has some limitations. As there is relatively scarce research on the physiological values of the blood of sheep and lambs in Turkey, we analyzed our results using globally recognized references (Byers and Kramer, 2011). This study was unable to examine findings resulting from geographical variations. Although volume of urine was not assessed in this study, it may be measured in future research. One finding of this study is that whey consumption may result in dehydration. Before advising the use of whey in warm and dry locations, the dehydration state should be thoroughly examined, and a future study should be designed on this topic. The increase in the hematocrit rate of the whey group at the beginning suggests that the association between whey use and dehydration should be studied in more detail. Further studies on the effects of whey on fat and sugar metabolism in sheep are recommended.

CONCLUSIONS

In conclusion, in terms of oxidative and inflammation parameters studied at the end of the experiment, no difference was observed between the two groups in the biochemical parameters of the effect. Although fluctuations were observed in some hematological parameters, there was not statistical difference between the groups in immune, oxidant, and antioxidant parameters. It is concluded that enriched whey drinking water showed promising effects on reducing oxidative stress parameters and why enriched drinking water can be used safely in fattening merino lambs.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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COMPLIANCE WITH ETHICAL STANDARDS

All animal manipulations were performed according to the EU Directive on the Protection of Animal Usage for Scientific Purposes (2010/63/EU). The research protocol was approved by the Ethical Committee of the Veterinary Control Central Research Institute (Approval Number: 2017/06).

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REFERENCES

Bilal T (2004) Laboratory diagnosis in veterinaray practice. İstanbul University Press, İstanbul (In Turkish).

Byers SR, Kramer JW (2011) Normal hematology of sheep and goats. In: DJ Weiss, KJ Wardrop (Eds.), Schalm's veterinary hematology. 6th ed, John Wiley Sons, New York: pp 836-842.

Danyer E, Bilal T, Altiner A, Aytekin İ, Atalay H (2021) The effect of vitamin E treatment on selected immune and oxidative parameters in Kivircik ewes suffering from transport stress. Journal of Animal Phy 105 (S1): 34-41.

Dayani O, Tahmasbi R, Khezri A, Sabetpay AR (2011) Effect of feeding dietary treated wheat straw with urea and whey on fattening lambs performance. Iranian Journal of Applied Animal Science 1 (4): 265-271.

DePeters EJ, Fisher LJ, Stone JL (1986) Effect of adding dried whey to starter diet of early and late weaned calves. Journal of Dairy Science 69 (1): 181-186.

Dinarello CA (1996) Biologic basis for interleukin-l in disease. The Journal of the American Society of Hematology 87 (6): 2095-2147.

El-Agamy EI (2007) The challenge of cow milk protein allergy. Small Ruminant Research 68 (1-2): 64-72.

Eseceli H, Esen S, Keten M, Altıner A, Bilal T (2021) Effect of whey protein-enriched water on performance and in vivo carcass measurements in fattening merino lambs. Alinteri Journal of Agricultural Sciences 36 (1): 61-65.

Fallah R (2019) Effects of adding whey and molasses on corn silage quality, growth performance and health of Simmental fattening calves. Journal of Livestock Science 10 (2):91-96.

Gabay C, Kushner I (1999) Acute-phase proteins and other systemic re-

sponses to infllammation. The New England Journal of Medicine 340 (6): 448-454.
Galloway DL, Goetsch AL, Forster LA, Brake AC, Johnson Z B (1993) Digestion, feed intake, and live weight gain by cattle consuming bermudagrass and supplemented with different grains. Journal of Animal Science71 (5): 1288-1297.
Gandhi K, Devi S, Gautam PB, Sharma R, Mann B, Ranvir S, Sao K, Pandey V (2019) Enhanced bioavailability of iron from spray dried whey protein concentrate-iron (WPC-Fe)complex in anaemic and weaning conditions. Journal of Functional Foods 58 (March): 275-281.
Giráldez FJ, Santos N, Santos A, Valdés C, López S, Andrés S (2021) Fattening lambs with divergent residual feed intakes and weight gains: unravelling mechanisms driving feed efficiency. Animal Feed Science and Technology 273 (2021):114821.
Hassan T, Abdel-Fattah F, Farid A, Kamel E. (2016) Effect of feeding guava waste on growth performance, diet digestibility, carcass characteristics and production profitability of ossimi lambs. Egyptian Journal of Nutrition and Feeds 19 (3): 461-472.
Håversen L, Ohlsson BG, Hahn-Zoric M, Hanson LÅ, Mattsby-Baltzer I (2002) Lactoferrin down-regulates the LPS-induced cytokine production in monocytic cells via NF-κB. Cellular Immunology 220 (2): 83-95.
Hoffman JR, Falvo MJ (2004) Protein - which is best? Journal of Sports Science and Medicine3 (3): 118-130.
Hulmi JJ, Lockwood CM, Stout JR (2010) Effect of protein/essential amino acids and resistance training on skeletal muscle hypertrophy: A case for whey protein. Nutrition and Metabolism 7: 28-31.
Kushibiki S, Hodate K, Kurisaki J, Shingu H, Ueda Y, Watanabe A, Shinoda M (2001) Effect of β-lactoglobulin on plasma retinol and triglyceride concentrations, and fatty acid composition in calves. Journal of Dairy Research 68 (4): 579-586.
Margaritelis NV, Chatzinikolaou PN, Bousiou FV, Malliou VJ, Papadopoulou SK, Potsaki P, Theodorou AA, Kyparos A, Geladas ND, Nikolaidis MG, Paschalis V (2020) dietary cysteine intake is associated with blood glutathione levels and isometric strength. International Journal of Sports Medicine 42 (05): 441-447.
Marnett LJ (1999) Lipid peroxidation - DNA damage by malondialdehyde. Mutation Research 424: 83-95.
Mattsby-Baltzer I, Roseanu A, Motas C, Elverfors J, Engberg I, Hanson, L A Å (1996) Lactoferrin or a fragment thereof inhibits the endotoxin-induced interleukin-6 response in human monocytic cells. Pediatr Res 40 (2): 257-262.
Mcgreal EP, Hearne K, Spiller OB (2012) Immunobiology off to a slow start : under-development of the complement system in term newborns is more substantial following premature birth. Immunobiology 217 (2): 176-186.
Nkosi BD, Meeske R (2010) Effects of whey and molasses as silage additives on potato hash silage quality and growth performance of lambs. South African Journal of Animal Sciences 40 (3): 229-237.
Pal S, Radavelli-Bagatini S (2013) The effects of whey protein on cardiometabolic risk factors. Obesity Reviews 14 (4): 324-343.
Prasad K (2006) C-Reactive Protein (CRP)lowering agents. Cardiovascular Drug Reviews 24 (1): 33-50.
Rapetti L, Falaschi U, Lodi R, Vezzoli F, Tamburin, A, Greppi GF, Enne G (1995) The effect of liquid whey fed to dairy goats on milk yield and quality. Small Ruminant Research 16 (3): 215-220.
Ribadeau-Dumas B, Grappin R (1989) Milk protein analysis. Le Lait 69 (5): 357-416.
Rokka S, Korhonen H, Nousiainen J, Marnila P (2001) Colostral whey concentrate supplement increases complement activity in the sera of neonatal calves. Journal of Dairy Research 68 (3): 357-367.
Ronghui S (2016) The reasearch on the anti-fatigue effect of whey protein powder in basketball training. The Open Biomedical Engineering Journal 9 (1): 330-334.
Rutherfurd KJ, Gill HS (2000) Peptides affecting coagulation. British Journal of Nutrition, 84 (Suppl. 1): S99-S102.
Schingoethe DJ (1976) Whey utilization in animal feeding: a summary and evaluation. Journal of Dairy Science, 59 (3) 556-570.
Susmel P, Spanghero M, Mills CR, Stefanon B (1995) Rumen fermentation characteristics and digestibility of cattle diets containing different whey:maize ratios. Animal Feed Science and Technology, 53 (1): 81-89.
Thivend P (1978) Use of whey in feeding ruminants. World Animal Review (12):20-24.
Tokajuk A, Zakrzeska A, Chabielska E, Car H (2019) Whey protein concentrate limits venous thrombosis in rats. Applied Physiology, Nutrition and Metabolism 44 (8): 907-910.
Wang H, Zhong X, Shi W, Guo B (2011) Study of malondialdehyde (MDA) content , superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities in chickens infected with avian infectious bronchitis virus. African Journal of Biotechnology 10 (45): 9213-9217.
Wang J, Radics G, Whelehan M, O'Driscoll A, Healy AM, Gilmer JF, Ledwidge M (2018) Novel iron-whey protein microspheres protect gut epithelial cells from iron-related oxidative stress and damage and improve iron absorption in fasting adults. Acta Haematologica 138 (4): 223-232.
Xu R, Liu N, Xu X, Kong B (2011) Antioxidative effects of whey protein on peroxide-induced cytotoxicity. Journal of Dairy Science 94 (8): 3739-3746.
Zhao Y, Weaver AC, Fellner V, Payne RL, Kim SW (2014) Amino acid fortified diets for weanling pigs replacing fish meal and whey protein concentrate: Effects on growth, immune status, and gut health. Journal of Animal Science and Biotechnology 2014 (57): 1-10.
ZoBell DR, Okine EK, Olson KC, Wiedmeier RD, Goonewardene LA, Stonecipher C (2005) Effects of feeding wheat straw and middlings ensiled with whey on digestibility and growth of cattle. Canadian Journal of Animal Science 85 (1): 69-74.