

## RESEARCH ARTICLE

## Evaluation of Homocysteine Levels in Neonatal Calves with Diarrhea

Süleyman KOZAT <sup>1,a</sup> (\*) Cumali ÖZKAN <sup>1,b</sup> Eda Nur OKMAN <sup>1,c</sup><sup>1</sup> Department of Internal Medicine, Faculty of Veterinary Medicine, University of Yuzuncu Yil, TR-65080 Van - TURKEY  
ORCIDs: <sup>a</sup> 0000-0001-5089-2623; <sup>b</sup> 0000-0001-8502-6987; <sup>c</sup> 0000-0001-9016-9739

Article ID: KVFD-2020-24894 Received: 26.08.2020 Accepted: 24.05.2021 Published Online: 26.05.2021

## Abstract

The aim of this study was to investigate the relationship between serum homocysteine (HCY), and creatinine, urea, venous blood gas and electrolytes values in neonatal calves with diarrhea. The study was conducted on a total of 30 calves, 20 with diarrhea and 10 healthy (control), with diarrhea complaints, of different races, sexes and ages ranging from 2-24 days. According to the venous blood gas results, the pCO<sub>2</sub> and base deficit values of calves with diarrhea were significantly higher (P<0.001) compared to the control group values, while pH, pO<sub>2</sub> and HCO<sub>3</sub> values were significantly lower (P<0.001). While serum Na<sup>+</sup> and Cl<sup>-</sup> concentrations in diarrheic calves did not show any statistical change when compared to the control group (P>0.05), serum K<sup>+</sup> concentrations were statistically higher (P<0.001). Serum HCY, folate and vitamin B<sub>12</sub> concentration values of diarrheic calves were significantly higher (P<0.001) when compared to the control group. As a result; in neonatal calves with diarrhea, it has been concluded that homocystein excretion is disrupted by low renal excretion due to decrease in glomerular filtration rate that caused hyperhomocysteine. In addition, it is thought that this study will shed light on studies that will reveal the effect of hyperhomocysteinemia in the cardiovascular system in diarrheic calves.

**Keywords:** Homocysteine, Neonatal calf, Diarrhea

## Yenidoğan İshalli Buzağılarda Homosistein Düzeylerinin Değerlendirilmesi

## Öz

Bu çalışmanın amacı ishalleri yenidoğan buzağılarda serum homosistein (HCY) ile kreatinin, üre, venöz kan gazı ve elektrolit değerleri arasındaki ilişkiyi araştırmaktır. Araştırma, ishal şikayeti olan, farklı ırk ve cinsiyette ve yaşları 2-24 gün arasında değişen 20 ishalleri ve 10 sağlıklı (kontrol) olmak üzere toplam 30 buzağı üzerinde yürütüldü. Venöz kan gazı sonuçlarına göre, ishalleri buzağuların pCO<sub>2</sub> ve baz açığı değerleri kontrol grubu değerlerine göre önemli (P<0.001) düzeyde yüksek belirlenirken, pH, pO<sub>2</sub> ve HCO<sub>3</sub> değerleri anlamlı (P<0.001) düzeyde düşük saptandı. İshalleri buzağılarda Na<sup>+</sup> ve Cl<sup>-</sup> elektrolit konsantrasyonlarının seviyeleri kontrol grubuna göre anlamlı bulunmazken, serum K<sup>+</sup> elektrolit konsantrasyon seviyeleri kontrol grubuna göre önemli düzeyde (P<0.001) yüksek tespit edildi. İshalleri buzağuların serum HCY, folat ve vitamin B<sub>12</sub> konsantrasyon değerleri kontrol grubuna göre anlamlı olarak yüksek (P<0.001) bulundu. Sonuç olarak; yenidoğan ishalleri buzağılarda sıvı kaybına bağlı olarak dolaşımdaki sıvı hacmi azalmakta ve böbreklerin glomüler filtrasyon hızı düşmektedir. Böbreklerde düşen glomüler filtrasyon hızına bağlı olarak homosisteinin böbrek yoluyla atılımı aksamakta ve sonucunda da hiperhomosisteine neden olmaktadır. Ayrıca ishal vakalarında artan homosistein konsantrasyonlarının kardiyovasküler sistemde bozukluklara neden olup olmadığına yönelik yapılacak araştırmalara ışık tutacaktır.

**Anahtar sözcükler:** Homosistein, Yenidoğan buzağı, İshal

## INTRODUCTION

Calf scour remains one of the most important problems faced by livestock industry <sup>[1]</sup> despite all the developments in the field of prophylaxis and treatment of calf scour. It is still common today and is directly related to deaths; it also leads to indirect economic losses with development pause and treatment expenses <sup>[2,3]</sup>. It is reported that diarrhea

affects 90-100% of neonatal calves in some livestock farming <sup>[1]</sup>, diarrhea related death in calves is more common than those of other reasons and causes great economic losses in Turkey livestock economy. The loss of fluid and electrolyte in diarrhea occurs mainly from the extracellular compartment in the body (intravascular + extravascular fluid) and as a result dehydration develops <sup>[2]</sup>. High fluid loss causes a decrease in plasma volume (hypovolemia),

## How to cite this article?

**Kozat S, Özkan C, Okman EN:** Evaluation of homocysteine levels in neonatal calves with diarrhea. *Kafkas Univ Vet Fak Derg*, 27 (3): 271-277, 2021. DOI: 10.9775/kvfd.2020.24894

## (\*) Corresponding Author

Tel: +90 533 5525466

Fax: +90 432 2251127

E-mail: skozat@hotmail.com (S. Kozat)



This article is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

hemoconcentration and low blood pressure. Decreased arterial blood pressure leads to decreased renal function and prerenal azotemia and H<sup>+</sup> excretion, on the other hand, increase anaerobic metabolism with decreased tissue perfusion [1-3].

Homocysteine (HCY) is a sulfurous amino acid that does not participate in proteins formed by methionine metabolism [4-7]. Plasma HCY is metabolised by remethylation and transsulfuration mechanisms [5,8]. S-adenosyl homocysteine (SAH) is formed by using S-adenosylmethionine (SAM) as a methyl donor by transmethylation. By hydrolyzing SAH, homocysteine and adenosine are formed [9]. Homocysteine is converted to methionine by remethylation where N5-methyltetrafolate is the methyl donor and vitamin B<sub>12</sub> is cofactor [6]. In the remethylation pathway, methionine synthase and N5-methyltetrafolate reductase enzymes act as catalysts. In addition, remethylation takes place in the liver and kidney by using the betaine methyl donor and the betaine-homocysteine methyltransferase enzyme, independent of vitamin B<sub>12</sub> and folic acid [10]. Vitamin B<sub>6</sub> is used as a cofactor in the transsulfuration pathway in the kidneys. Homocysteine is combined with serine and cystathionine is synthesized with the enzyme B-cystathionine synthase. Cystathionine is also converted into cysteine with the enzyme cystathionase. Cysteine is either excreted directly from the kidneys or converted into glutathione and sulfate [4,6,11].

Increases in plasma total homocysteine levels are reported to be caused by N5-methyltetrahydrofolate reductase and cystathionine B synthetase enzyme defects, or dietary deficiencies of enzyme cofactors B<sub>12</sub>, B<sub>6</sub> and folic acid [6]. Plasma homocysteine levels increase with creatine due to renal failure [12]. The increase in total homocysteine levels, ie, hyperhomocysteinemia, has been shown to cause cardiovascular system disease as a result of its role in atherogenesis, atherosclerosis and thrombosis [10]. Hyperhomocysteinemia causes direct vascular endothelial damage, changes the anticoagulant effect of the endothelium to procoagulant and causes proliferation in smooth muscle cells [10,13]. Present information clarifies that the accurate renal function play basic role in homocysteine clearance. This witnessing and the reverse connection between homocysteine amounts and glomerular filtration rate (GFR) signifies that the kidneys play important role in HCY metabolism [6]. Although plasma folic acid levels are normal in kidney failure in many studies, decreasing plasma total homocysteine levels with folic acid supplementation have increased the possibility of relative intracellular vitamin deficiency [14]. This situation is reported to be related to the disorder in the conversion of folate to metabolically appropriate form [6]. Homocysteine is an intermediate of methionine metabolism. Hyperhomocysteinemia (HHCY) can be caused by a deficiency in enzymes or vitamin cofactors required for HCY metabolism, or from kidney disease [4,6,12,14].

Research has shown that there is a close relationship between plasma HCY and GFR. HCY is cleared from the body by urinary excretion after glomerular filtration, just like creatinine. It has been presented that kidney plays a major role in the maintenance of HCY plasma homeostasis in rats [15]. Decreased kidney function in chronic uremic patients, which is one of the causes of hyperhomocysteinemia, is an important determinant for plasma homocysteine concentration and there is a close relationship between decreased kidney reserve and homocysteine levels [14]. Homocysteine levels increase at least three to four times in patients with renal failure compared to normal individuals [16].

In this study was investigated the relationship between serum HCY and creatinine, urea, venous blood gas and electrolyte values in newborn calves with diarrhea. Fluid volume and glomerular filtration rate decreases as a result of dehydration in neonatal calves with diarrhea. The homocysteine transsulfuration mechanism is thought to be impaired as a result of the decreased GFR. Based on this equation, homocysteine will cause an increase in the total plasma homocysteine level as a result of disruption of transsulfuration mechanism in homocysteine. It was aimed to reveal whether there is a change in homocysteine levels in dehydrated calves, especially due to decreased kidney function.

## MATERIAL AND METHODS

This research was approved (28/12/2017 and 27552122-604.01.02-E.91873) by the Animal Research Ethical Committee of Van Yuzuncu Yil University, Local Ethics Committee.

### Animal

The animal material of this experiment consisted of 2-24 days old 30 calves from different races and genders brought to the Veterinary Faculty of Animal Hospital. Thirty calves were divided into 2 groups as twenty calves were diarrheic and ten calves were healthy (control). All calves in the control and experimental groups were examined on physical examination. The diarrheic calves were assessed by evaluating; defecation frequency (>4 times per day), fecal consistency (color and content), intensity (mucous, bloody an abundant), body temperature, heart frequencies, skin elasticity, position of the eye in the orbit, body retention, suction reflex examination and duration of diarrhea [17]. Calves were classified as having diarrhea after being evaluated according to clinical examination.

### Laboratory Analysis

Blood samples were taken from *v. jugularis* in accordance with the technique for the analysis of hematological and biochemical parameters in diarrheic calves and healthy calves.

### Hematological Analysis

In order to analyse the hematological parameters, blood samples were taken from *v. jugularis* into anticoagulated tubes (3 mL EDTA) in accordance with the blood samples technique. Hemoglobin concentration (HB), hematocrit value (HCT), leukocyte count (LEU), erythrocytes (RBC), platelet count (PLT), lymphocytes (LYM), monocytes (Mon), neutrophil (NEU), eosinophil (EO) and mean corpuscular hemoglobin concentration (MCHC) values were measured from the blood samples taken in anticoagulated tubes with the Veterinary Hemogram device (Veterinary MS4-s-Melet Schloesing Laboratories in France).

### Blood Gas Analysis

Whole blood from the vena jugularis of the calves with dry lithium heparin content (2 mL, 22G × 11 / 4) were taken for blood gas analysis and blood gas analysis were performed for 3 min. Blood gases (pH, pCO<sub>2</sub>, pO<sub>2</sub>, HCO<sub>3</sub> and base deficit), Na<sup>+</sup> (mmol/L), K<sup>+</sup> (mmol/L) and Cl<sup>-</sup> (mmol/L) concentrations of diarrheic and healthy calves were measured with the blood gases analyser (Radiometer ABL80, Denmark).

### Biochemical Analysis

Blood samples were taken from *v. jugularis* to gel biochemical serum tubes (10 mL) to analyze biochemical parameters. Serum tubes were centrifuged for 15 min at 3000 rpm (rate per minute) and serum sample were taken into eppendorfs. The serum samples were kept at -20°C until biochemical parameters analysis. In serum samples obtained, total protein, albumin, urea and creatinine levels of diarrheic and healthy calves were measured with the autoanalyzer (BS-120 Vet-Mindray, China) device. Serum homocysteine levels of diarrheic and healthy calves were measured using the ELISA device (Biotek Elx800) as specified in the procedure of the homocysteine commercial test kits (Axis® Homocysteine EIA-UK). Serum vitamin B<sub>12</sub> and folate levels of diarrheic and healthy calves were measured with the autoanalyzer (Elecyc 2010 Roche Hitachi-Japan).

### Statistical Analysis

Descriptive statistics for the results were expressed as mean and standard deviation. Independent samples t-test was used to compare two groups (diarrhea and healthy). Pearson Correlation was used to some variables in calves with diarrhea. SPSS 21 (IBM, USA) package program was used for necessary statistical analysis.

## RESULTS

### Hematological Findings

In the statistical analysis; WBC, HCT, EO MCV, MCH and HB values of calves with diarrhea were significantly higher than those of the control group (P<0.001) and PLT value was found to be significantly lower (P<0.001) (Table 1).

### Biochemical Findings

In the statistical analysis of biochemical parameters; urea, creatinine and total protein levels of diarrheic calves were significantly higher when compared with the same parameters of the control group (P<0.001, P<0.05) (Table 2).

According to the statistical analysis; while the pCO<sub>2</sub> value of calves with diarrhea was found to be significantly higher compared to the control group (P<0.001), pH, pO<sub>2</sub>, HCO<sub>3</sub> and base deficit values of calves with diarrhea were significantly lower than those of the control group (Table 3).

The concentrations of Na<sup>+</sup> and Cl<sup>-</sup> electrolytes in calves with diarrhea were not significantly different from than those of the control group, however, the concentration levels of K<sup>+</sup> in diarrheic calves were significantly higher compared to the control group (P<0.001) (Table 4).

**Table 1.** Hematological parameters in healthy calves and dehydrated calves with diarrhoea

Parameter	Healthy Calves (n=10) X ± SD	Diarrhoeic Calves (n=20) X ± SD	P Value
Age (day)	12.80±4.32	12.90±5.54	0.374
WBC (m/mm <sup>3</sup> )	10.37±1.03	15.86±7.39*	0.028
RBC (m/mm <sup>3</sup> )	9.01±0.90	8.87±1.79	0.822
HCT (%)	28.36±2.73	40.07±4.94*	0.001
LYM (m/mm <sup>3</sup> )	6.92±1.49	7.89±5.76	0.608
MON (m/mm <sup>3</sup> )	0.58±0.11	0.76±0.41	0.164
NEU (m/mm <sup>3</sup> )	4.53±1.34	5.55±3.76	0.415
EO (m/mm <sup>3</sup> )	0.38±0.13	0.84±0.69*	0.049
PLT (m/mm <sup>3</sup> )	699.70±278.35	336.95±169.83*	0.001
MCV (fl)	29.06±3.151	34.23±3.77*	0.001
MCH (pg)	12.31±1.36	13.61±1.49*	0.028
HB (g/dL)	10.11±1.23	12.79±2.66*	0.005

WBC: White blood cell; RBC: red blood cell; HCT: hematocrit; LYM: lymphocyte; MON: monocyte; NEU: neutrophil; EO: eosinophil; PLT: platelet; MVC: mean corpuscular volume; MCH: mean corpuscular hemoglobin; HB: hemoglobin; \* Means in the same row with different superscripts are significantly different (P<0.05)

**Table 2.** Biochemical parameters in healthy calves and dehydrated calves with diarrhoea

Parameter	Healthy Calves (n=10) X ± SD	Diarrhoeic Calves (n=20) X ± SD	P Value
Albumin (g/dL)	2.64±0.64	2.88±0.36	0.293
TP (g/dL)	5.81±0.96	6.80±1.10*	0.021
Creatinine (mg/dL)	0.74±0.07	2.68±1.40*	0.001
Urea (mg/dL)	17.03±5.87	62.87±22.35*	0.001

TP: total protein; \* Means in the same row with different superscripts are significantly different (P<0.05)

**Table 3.** Blood gas parameters in healthy calves and dehydrated calves with diarrhoea

Parameter	Healthy Calves (n=10) X ± SD	Diarrhoeic Calves (n=20) X ± SD	P Value
pH	7.33±0.05	7.15±0.09*	0.001
pCO <sub>2</sub> (mm/Hg)	33.94±3.91	39.37±4.28*	0.002
pO <sub>2</sub> (mm/Hg)	39.90±4.63	28.40±6.51*	0.001
HCO <sub>3</sub> (mmol/L)	21.66±4.67	15.57±6.19*	0.011
Base deficit (mmol/L)	18.01±3.02	-1.08±8.23*	0.001

pO<sub>2</sub>: partial pressure of oxygen; pCO<sub>2</sub>: partial pressure of carbon dioxide; \* Means in the same row with different superscripts are significantly different (P<0.05)

**Table 4.** Serum electrolyte concentrations in healthy calves and dehydrated calves with diarrhoea

Parameter	Healthy Calves (n=10) X ± SD	Diarrhoeic Calves (n=20) X ± SD	P Value
Na <sup>+</sup> (mmol/L)	137.20±3.01	134.10±7.75	0.236
K <sup>+</sup> (mmol/L)	4.99±0.25	6.55±1.23*	0.001
Cl <sup>-</sup> (mmol/L)	99.90±4.70	97.60±8.10	0.411

\* Means in the same row with different superscripts are significantly different (P<0.05)

**Table 5.** Homocysteine, vitamin B<sub>12</sub> and folate concentrations in healthy calves and dehydrated calves with diarrhoea

Parameter	Healthy Calves (n=10) X ± SD	Diarrhoeic Calves (n=20) X ± SD	P Value
HCY (µmol/L)	0.87±0.22	1.43±0.32*	0.001
Vitamin B <sub>12</sub> (pg/mL)	149.57±28.58	248.50±101.41*	0.006
Folate (ng/mL)	4.66±3.28	12.08±7.61*	0.007

HCY: homocysteine; \* Means in the same row with different superscripts are significantly different (P<0.05)

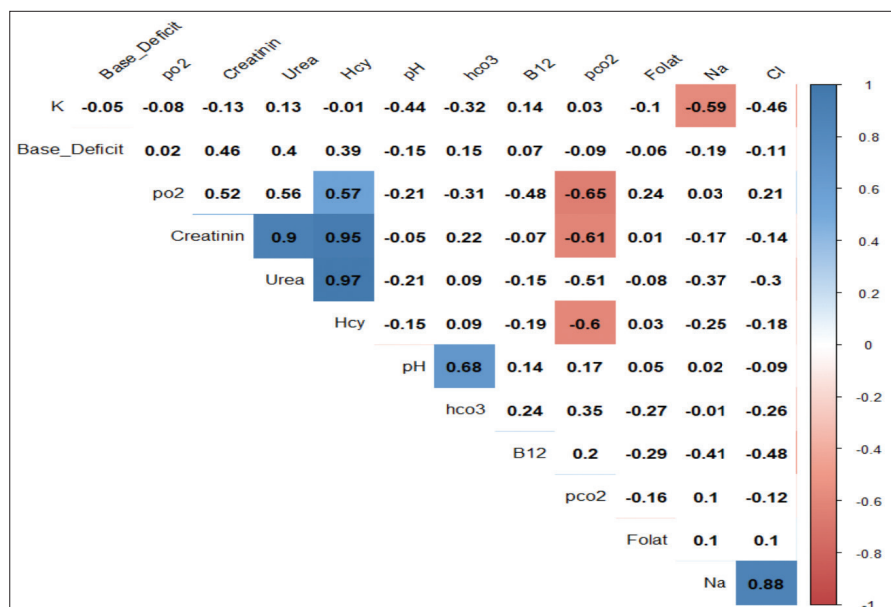
Serum homocysteine, folate, and vitamin B<sub>12</sub> concentration values of diarrheic calves were significantly higher (P<0.001) compared to the control group (Table 5).

In the statistical analysis of the correlations of some variables of neonatal calves with diarrhea, a positive correlation was found between serum HCY, creatinine, and urea levels (r=0.95, P<0.001; r=0.97, P<0.001, respectively) (Fig. 1).

## DISCUSSION

It has been reported that significant changes in hematological parameters are detected depending on the degree of dehydration in fluid losses due to diarrhea [2,17-19]. There is a positive relationship between the degree of dehydration and Hct value in calves with diarrhea and the severity of dehydration can be determined from the HCT value [2]. Increased HCT value, RBC count, WBC, NEU count and HB values have been reported in neonatal calves with diarrhea [1,2]. It is reported that it occurs as a result of the reaction of the body against leukocytosis, gastrointestinal infection in cases with diarrhea [1]. In this study, WBC, HCT, EO, MCV and MCH values of diarrheic calves were found to be significantly higher when compared with the same parameters of the control group (P<0.001), while the PLT value was found to be significantly lower. Some researchers [1,20] reported changes in hematological parameters in calves with diarrhea. Also in this study, increases in MCV and MCH results from the increase in hemoglobin concentration.

In many studies, in general, a decrease in bicarbonate concentration in calves with diarrhea is considered as a marker of acidosis [21]. In calves with diarrhea, metabolic acidosis initially results in bicarbonate with feces, as well as the presence of unidentified organic acids in plasma [22,23] and a decrease in glomerular filtration rate in response to



**Fig 1.** Correlation of some variables in calves with diarrhea. Light and dark blue colors indicate positive correlation among parameters. Light and dark red colors indicate negative correlation among parameters

severe dehydration [18]. In calves with diarrhea,  $\text{pH} < 7.28$  and  $\text{HCO}_3^-$  concentration of 20.0 mmol/L are reported, which reflects metabolic acidosis [3,19]. In another study, researchers found the mean base excess to calves with 4-8% dehydrated and moderately metabolic acidosis:  $-9.6$  mEq/L, average  $\text{pCO}_2$ : 43 mmHg, average  $\text{pO}_2$ : 39 mmHg and average  $\text{pH}$ : 7.21 [24]. In this study,  $\text{pH}$  of calves with diarrhea was determined as  $7.15 \pm 0.10$ ,  $\text{pCO}_2$ :  $39.37 \pm 4.28$ ,  $\text{pO}_2$ :  $28.40 \pm 6.51$ ,  $\text{HCO}_3^-$ :  $15.57 \pm 6.19$  and base deficit:  $-1.08 \pm 8.23$  mmol/L. The values of the blood gas parameters obtained in our study are similar to the findings of many researchers [3,18,19,24].

In calves with diarrhea, arterial blood pressure and glomerular filtration rate (GFR) decreases as a result of hypovolemia [2,19,25], urine and creatinine retention increase, and serum concentrations of these nitrogenous substances increase [2]. In this study; while control group calves had urea:  $17.03 \pm 5.87$  mg/dL and creatinine:  $0.74 \pm 0.07$  mg/dL, calves with diarrhea were determined to have urea:  $62.87 \pm 22.35$  mg/dL and creatinine:  $2.68 \pm 1.40$  mg/dL. However, urea and creatinine levels of calves with diarrhea were significantly higher than the control group ( $P < 0.001$ ). The urea and creatinine data of calves with diarrhea support the data of the previous studies [2,21]. The buffering required eliminating diarrhea-induced acidemia results in hyperkalemia as a result of  $\text{K}^+$ . Hyperkalemia results from the direct electrochemical exchange of potassium for protons across the cell membrane [2,18]. Therefore,  $\text{H}^+$  in the extracellular fluid enters the intracellular fluid, whereas  $\text{K}^+$  in the intracellular fluid enters the extracellular fluid [19]. Strongest relations were described between plasma  $\text{K}^+$  and indices dehydration, emphasizing the importance of decreased glomerular filtration rate for the development of hyperkalemia [2,19,21]. In this study, the increase in serum  $\text{K}^+$  concentrations in calves with diarrhea supports the researchers' data and hypotheses [2,19,25].

Several studies in different diseases have been conducted in the fields of human and veterinary medicine regarding the plasma and serum homocysteine [5,9-15,26]. However, there was no research on whether there was a change in HCY concentrations in patients with fluid loss due to diarrhea. There is a direct relationship between homocysteine and reduction in glomerular filtration and serum creatinine reduction in kidneys impairment. Creatinine concentration level in individuals is between 150-500  $\mu\text{mol/L}$ , typically homocysteine values are between 20-30  $\mu\text{mol/L}$  in human medicine [27]. In kidney dysfunction, this mechanism does not work and accordingly an increase in the total amount of homocysteine in plasma is observed [10]. Hyperhomocysteine is existent in individuals with declining kidney function, so that this alteration can be considered as a major factor in the progression of kidney diseases [28]. Vascular complications related to hyperhomocysteinemia may also occur in uremic pediatric patients [26]. There is highly correlated GFR ( $r = -0.70$ ,  $P < 0.001$ ) plasma homo-

cysteine as a result of decreased kidney function in moderate renal failure [29]. In determining GFR, serum homocysteine level plays a key role in early diagnosis of kidney diseases compared to serum creatinine level [16,29]. Serum creatinine and blood urea nitrogen measurements are easy for indirect symptoms of GFR [30]. Increased protein catabolism due to fasting, other than prerenal insufficiency, exacerbates the increase in serum urea concentration in calves with diarrhea [2]. In a study, it has been reported that there is a positive correlation between plasma homocysteine concentration and creatinine level and negative correlation with creatinine clearance [14]. In this study, a positive correlation was found between creatinine levels and HCY concentrations of diarrheic calves. Serum HCY concentration levels were high in cases with high creatinine concentrations. These data support the data of many researchers who reveal the relationship between creatinine, HCY and kidney function [14,27-29].

Other amino acids and intermediates involved in homocysteine metabolism are also affected in patients with uremia. Patients have normal levels of methionine, whereas transsulfuration metabolites such as cystationin and cysteine are high. It has been shown that plasma concentration of intermediates such as S-adenosyl methionine (Ado-Met) and S-adenosyl homocysteine (Ado-HCY) increase, the increase in Ado-HCY is more pronounced, and accordingly the Ado-Met/Ado-HCY ratio decreases. Increased plasma and erythrocyte homocysteine in uremic patients have been found to cause the accumulation of Ado-HCY, a toxic component in erythrocytes [16]. It is stated that plasma cysteine-homocysteine mixed disulfide levels have a positive correlation between creatinine levels in patients with renal failure [26]. In our study, the increase in HCY concentrations of diarrhea calves and the higher vitamin  $\text{B}_{12}$  and folate levels compared to the control group supports the data of the researchers [16,26]. Plasma HCY concentrations did not decrease although homocysteine metabolism increased in favor of transsulphuration due to the disruption of remethylation pathway in uremic patients [15,16]. In many studies, plasma HCY levels have been reported to be significantly increased in patients with moderate renal impairment and HCY level has increased significantly in the last stage of kidney disease [11]. An increase in plasma HCY level in kidney diseases may result from an increased production rate (i.e. transmethylation), a reduced rate of extraction from transsulfation or remethylation, or a decrease in HCY excretion [31]. In human medicine, HCY is used to evaluate complications of kidney, cardiovascular and other diseases [11,15,26]. HCY has been shown to be an independent risk factor for cardiovascular disease in kidney patients. It has been reported that there is a positive correlation between plasma HCY levels and GFR and serum creatinine [32]. Current evidence suggests that the main mechanism for hyperhomocysteinemia in kidney failure is a reduction in HCY removal from the body. However, it

is argued whether the increase in homocysteine level is a result of a decrease in kidney metabolic clearance or a result of extrarenal metabolic changes [31]. Kidneys play a major role in removing a large number of aminotiol or HCY-related compounds from the circulation (i.e. cysteine-glycine, glutathione, AdoMet and AdoHCY) in human medicine [15]. However, the glomerular filtration of HCY appears to be limited due to protein binding. Besides of the glomerular filtration, it can remove normal kidney HCY by plasma flow and peritubular uptake. Although it is in the absolutely low normal range, if it is associated with HCY levels in uremia, flow decreases along the transsulfuration path; In addition, the remethylation path is impaired and HCY increases in mild to moderate renal failures [31]. Because there is a tight relationship between plasma flow and GFR, and decreasing GFR causes an increase in plasma HCY levels [11]. In this study, urea, creatinine and HCY levels of diarrheic calves were found to be significantly higher than those of the control group. In addition, in the statistical analysis of the correlations of some variables of diarrheic calves, positive correlation was found between HCY and creatinine and urea concentrations (respectively  $r=0.95$ ,  $P<0.001$ ;  $r=0.97$ ,  $P<0.001$ ). It is known that the increase in HCY levels in calves with diarrhea may be due to decreased glomerular filtration. The study supports the hypotheses suggested by the researchers [11,15,31,32] in the increase of HCY levels of the related diarrheic calves.

As a result, in this study, significant changes were detected in urea and creatinine concentrations, which are important markers in the evaluation of glomerular filtration rate or kidney function in diarrheic calves. Hyperhomocysteinemia caused by glomerular filtration rate of kidneys decreases resulting from fluid volume decreases as a result of fluid loss in neonatal calves with diarrhea. The increase in HCY level in calves with diarrhea; HCY may be caused by an increased production rate (i.e. transmethylation), reduction from remethylation or transsulfuration rate of homocysteine, or a decrease in HCY excretion. It is concluded that hyperhomocysteinemia in diarrhea cases will shed light on the studies to be conducted about whether it causes disorders in the cardiovascular system.

## CONFLICT OF INTEREST

The authors have no competing or conflict of interests in submitting this article.

## FINANCIAL SUPPORT

This study was supported by the Scientific Research Project Directory of the University of Yuzuncu Yil, Van, Turkey (Project No. TSA-2019-7946). We would like to thank to the Scientific Research Directory of the University of Yuzuncu Yil for their financial support on this study.

## AUTHOR CONTRIBUTIONS

SK and CÖ designed the project. ENO provided samples. SK, CÖ and ENO performed statistical analysis of data. All authors also contributed to the preparation of the manuscript.

## REFERENCES

1. Shekhar S, Ranjan R, Singh CV, Kumar P: Prevalence, clinicohaemato-biochemical alterations in colibacillosis in neonatal calves. *Int J Curr Microbiol Appl Sci*, 6 (9): 3192-3198, 2017. DOI: 10.20546/ijcmas.2017.609.393
2. Kozat S, Voyvoda H: İshalli buzağılarda kristalloid (laktatlı ringer) ve kolloid+kristalloid (%6 dekstran-70+ laktatlı Ringer) infüzyon solüsyonlarının rehidratasyon etkinliği. *Van Sağ Bil Derg*, 9, 139-151, 2006.
3. Zeybek S, Civelek T: The time dependent effects of intravenous administration of isotonic sodium bicarbonate on venous acid-base status and renal function in neonatal calves with acute diarrhea. *Kocatepe Vet J*, 7, 39-46, 2014. DOI: 10.5578/kvj.7375
4. Blom HJ, Smulders Y: Overview of homocysteine and folate metabolism. with special references to cardiovascular disease and neural tube defects. *J Inherit Metab Dis*, 34 (1): 75-81, 2011. DOI: 10.1007/s10545-010-9177-4
5. Çayır C, Kozat S: Investigation of homocysteine levels in healthy dogs. *J Vet Sci Anim Husband*, 4 (3): 305, 2016. DOI: 10.15744/2348-9790.4.305
6. Kozat S, Okman EN: Homocystein: A new biochemical marker in livestock sector. *J Adv Vet Anim Res*, 4, 319-332, 2017. DOI: 10.5455/javar.2017.d230
7. Özkan C, Kozat S, Kaya A, Akgül Y: Serum homocysteine, vitamin B<sub>12</sub> and folate levels in healthy Turkish Van Cats. *J Adv Vet Anim Res*, 4, 58-64, 2017. DOI: 10.5455/javar.2017.d191
8. Bostom AG, Lathrop L: Hyperhomocysteinemia in end stage renal disease: Prevalence, etiology, and potential relationship to atherosclerotic outcomes. *Kidney Int*, 52, 10-20, 1997. DOI: 10.1038/ki.1997.298
9. Kılıçkap A, Kozat S: Research of serum homocysteine levels in healthy cows. *J Vet Sci Anim Husband*, 5 (1): 103, 2017. DOI: 10.15744/2348-9790.5.103
10. Kim J, Kim H, Roh H, Kwon Y: Causes of hyperhomocysteinemia and its pathological significance. *Arch Pharm Res*, 41, 372-383, 2018. DOI: 10.1007/s12272-018-1016-4
11. Amin HK, El-Sayed MIK, Leheta OF: Homocysteine as a predictive biomarker in early diagnosis of renal failure susceptibility and prognostic diagnosis for end stages renal disease. *Ren Fail*, 38 (8): 1267-1275, 2016. DOI: 10.1080/0886022X.2016.1209382
12. Stamler JS, Osborne JA, Jaraki O, Rabbani LE, Mullins M, Singel D, Loscalzo, J: Adverse vascular effects of homocysteine are modulated by endothelium-derived relaxing factor and related oxides of nitrogen. *J Clin Invest*, 91, 308-318, 1993. DOI: 10.1172/JCI116187
13. Temel İ, Özerol E: Homosistein metabolizma bozuklukları ve vasküler hastalıklarla ilişkisi. *İnönü Üniv Tıp Fak Derg*, 9, 149-157, 2002.
14. Chauveau P, Chadefaux B, Coude M, Aupetit J, Hannedouche T, Kamoun P, Jungers P: Hyperhomocysteinemia, a risk factor for atherosclerosis in chronic uremic patients. *Kidney Int Suppl*, 41, 72-77, 1993.
15. Long Y, Nie J: Homocysteine in renal injury. *Kidney Dis*, 2, 80-87, 2016. DOI: 10.1159/000444900
16. Van Guldener C, Stam F, Stehouwer CDA: Homocysteine metabolism in renal failure. *Kidney Int*, 59 (Suppl. 78): S234-S237, 2001. DOI: 10.1046/j.1523-1755.2001.59780234.x
17. Gultekin M, Voyvoda H, Ural K, Erdogan H, Balıkcı C, Gultekin G: Plasma citrulline, arginine, nitric oxide, and blood ammonia levels in neonatal calves with acute diarrhea. *J Vet Intern Med*, 33, 987-998, 2019. DOI: 10.1111/jvim.15459
18. Constable PD, Stampfli HR, Navetat H, Berchtold J, Schelcher F:

Use of a quantitative strong ion approach to determine the mechanism for acid-base abnormalities in sick calves with or without diarrhoea. *J Vet Intern Med*, 19, 581-589, 2005. DOI: 10.1111/j.1939-1676.2005.tb02731.x

**19. Öcal N, Yasa Duru S, Yağcı BB, Gazyağcı S:** İshalli buzağılarda asit-baz dengesi bozukluklarının saha şartlarında tanı ve sağaltımı. *Kafkas Univ Vet Fak Derg*, 12, 175-183, 2006.

**20. Özkan C, Akgül Y:** Neonatal ishallerde buzağılarda hematolojik, biyokimyasal ve elektrokardiyografik bulgular. *YYU Vet Fak Derg*, 15, 123-129, 2004.

**21. Constable PD:** Clinical assessment of acid-base status: Comparison of the henderson hasselbalch and strong ion approaches. *Vet Clin Pathol*, 29 (4): 115-128, 2000. DOI: 10.1111/j.1939-165X.2000.tb00241.x

**22. Erdogan H, Voyvoda H:** Effects of rapid and ultra-rapid fluid resuscitation guided by blood lactate clearance rate in diarrheic calves. *J Hellenic Vet Med Soc*, 70, 1413-1420, 2019. DOI: 10.12681/jhvms.20349

**23. Özaydın İ, Erkilic EE, Kılıç E, Yayla S, Baran V:** Yenidoğan buzağılarda sıvı-elektrolit sağaltımı. In, Baran V (Ed): Ruminantlarda Neonatal Cerrahi. Ankara, Türkiye Klinikleri, 105-110, 2019.

**24. Güzelbektas H, Çoskun A, Şen İ:** Relationship between the degree of dehydration and the balance of acid-based changes in dehydrated calves with diarrhoea. *Bull Vet Inst Pulawy*, 51, 83-85, 2007.

**25. Constable PD, Grünberg W:** Hyperkalemia in diarrheic calves: Implications for diagnosis and treatment. *Vet J*, 195, 271-272, 2013. DOI: 10.1016/j.tvjl.2012.11.002

**26. Merouani A, Genest J, Rozen, R, Lambert M, Mitchell GA, Dubois J,**

**Robitaille P:** Cerebral vascular complications in a cystinotic uremic child. *Pediatr Nephrol*, 13, 73-76, 1999. DOI: 10.1007/s004670050567

**27. Still RA, McDowell IF:** Clinical implications of plasma homocysteine measurement in cardiovascular disease. *J Clin Pathol*, 51, 183-188, 1998. DOI: 10.1136/jcp.51.3.183

**28. Martella BM, Veiga GRL, Alves BCA, Azzalis LA, Junqueira VBC, Gehrkei FS, Fonseca FLA, Bacci MR:** The importance of homocysteine levels in the prognosis of patients with chronic renal disease and in hemodialysis patients. *J Bras Patol Med Lab*, 54, 170-176, 2018. DOI: 10.5935/1676-2444.20180030

**29. Arnadotir M, Hultberg B, Nilsson-Ehle P, Thysel H:** The effect reduced glomerular filtration rate on plasma total homocysteine concentration. *Scand J Clin Lab Invest*, 56, 41-46, 1996. DOI: 10.3109/00365519609088586

**30. Braun JP, Lefebvre HP, Watson ADJ:** Creatinine in the dog: A review. *Vet Clin Pathol*, 32 (4): 162-179, 2003. DOI: 10.1111/j.1939-165X.2003.tb00332.x

**31. Garibotto G, Sofia A, Valli A, Tarroni A, Di Martino M, Cappelli V, Aloisi F, Procopio V:** Causes of hyperhomocysteinemia in patients with chronic kidney diseases. *Semin Nephrol*, 26 (1): 3-7, 2006. DOI: 10.1016/j.semnephrol.2005.06.002

**32. Rossi S, Rossi G, Giordano A, Paltrinieri S:** Homocysteine measurement by an enzymatic method and potential role of homocysteine as a biomarker in dogs. *J Vet Diagn Invest*, 20, 644-649, 2008. DOI: 10.1177/104063870802000520