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Original article

Evaluation of the clinical efficacy of racecadotril in the treatment of neonatal calves with infectious diarrhea

B. Tras[1], M. Ok[2], M. Ider[2], T.M. Parlak[1], R. Yildiz[3], H. Eser Faki[1], Z. Ozdemir Kutahya[4], K. Uney[1]

[1] Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Selcuk, Ardicli Neighborhood, 42100, Konya, Turkey
[2] Department of Internal Medicine, Faculty of Veterinary Medicine, University of Selcuk, Ardicli Neighborhood, 42100, Konya, Turkey
[3] Department of Internal Medicine, Faculty of Veterinary Medicine, University of Mehmet Akif Ersoy, Yakakoy, 15030, Burdur, Turkey
[4] Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Cukurova, Fatih Sultan Mehmet Avenue, 01930, Adana, Turkey

Abstract

Racecadotril, used as an antidiarrheal drug in humans and some animals such as the dog, inhibits peripheral enkephalinase, which degrades enkephalins and enkephalinase inhibition induces a selective increase in chloride absorption from the intestines. The study material consisted of 46 calves with infectious diarrhea and 14 healthy calves in the age 2-20 days. The calves were divided into eight groups; healthy calves (HG), healthy calves administered racecadotril (HRG), calves with *E.coli*-associated diarrhea (ECG), calves with *E.coli*-associated diarrhea administered racecadotril (ECRG), calves with bovine *Rotavirus/Coronavirus*-associated diarrhea (VG), calves with bovine *Rotavirus/Coronavirus*-associated diarrhea administered racecadotril (VRG), calves with *C. parvum*-associated diarrhea (CG) and calves with *C. parvum*-associated diarrhea administered racecadotril (CRG). Calves in the racecadotril groups received oral racecadotril at a dose of 2.5 mg/kg twice a day for 3 days. A routine clinical examination of all calves was performed. Hemogram and blood gas measurements were made from the blood samples. Standard diarrhea treatment was applied to the HG, ECG, CG, and VG groups. Clinical score parameters such as appetite, feces quality, dehydration, standing and death and some blood gas and hemogram parameters were evaluated to determine the clinical efficacy of racecadotril. Clinical score parameters were determined observationally. Blood gas measurements were performed using a blood gas analyzer. The hemogram was performed using an automated hematologic analyzer. Statistically significant differences were determined in the blood pH, bicarbonate, base deficit, lactate, and total leukocyte count in calves with diarrhea compared to healthy calves. After the treatments, these parameters were found to be within normal limits. At the end of treatment, 42 of the 46 diarrheal calves recovered, while 4 died. We found that racecadotril was effective in improving both clinical recovery and feces consistency in neonatal calves with diarrhea caused by *E. coli.* As a result, it can be stated that racecadotril, which has an antisecretory effect, is beneficial in the treatment of bacterial diarrhea caused by such as *E. coli.*

Keywords: neonatal calf, infectious diarrhea, racecadotril, treatment

Correspondence to: T.M. Parlak, e-mail: tugba.parlak@selcuk.edu.tr, tel.: +90553 388 46 44

Introduction

Calf deaths associated with diarrhea in the neonatal period are the most important problem of cattle breeding around the world. Neonatal calf diarrhea causes more economic losses than other diseases (Urie et al. 2018, Boranbayeva et al. 2020, Ok et al. 2020). Economic losses result from deaths, poor performance, growth retardation, loss of time and treatment costs (Ok et al. 2009, Sen et al. 2009, Coskun et al. 2010, Trefz et al. 2015).

The most common bacterial cause of neonatal calf diarrhea is enterotoxigenic *E. coli*, which is a major cause of economic loss in calves within the first 4 days after birth. *Bovine Rota* and *Coronavirus* cause diarrhea after the first week of birth in calves. *C. parvum* is also the most common protozoal cause of diarrhea in calves from 1 to 4 weeks of age (Naylor 2009, Ok et al. 2009, Sen et al. 2009, Coskun et al. 2010, Trefz et al. 2015, Ok et al. 2020). *E. coli,* which has different strains and produces an enterotoxin, causes diarrhea with distinct mechanisms. These mechanisms, which alter intestinal functions, are i) effacement of microvilli on the epithelial surface of the intestine, ii) rapid inactivation of sodium-D-glucose transporter, and iii) stimulation of adenylate cyclase enzyme. The latter mechanism, associated with enterotoxin, causes secretory diarrhea characterized by an excessive secretion of intestinal fluid. It is indicated that *E. coli* changes other epithelial events that indirectly affect ion transport, epithelial integrity, and immune responses in intestinal epithelial cells (Turgut and Ok 1997, Hodges and Gill 2010). Viruses and parasites cause loss of villi in the intestines and reduce the absorption of water-electrolytes from the intestines, causing osmotic diarrhea (Turgut and Ok 1997, Field 2003, Hodges and Gill 2010).

In recent years, racecadotril (acetorphan), a prodrug with an antisecretory effect, has been used to treat acute diarrhea. Racecadotril is used as an antidiarrheal drug in human medicine and dog. It inhibits peripheral enkephalinase, which breaks down enkephalins both *in vitro* and *in vivo* and the inhibition of enkephalinase causes a selective rise in chloride absorption from the intestines (Bergmann et al. 1992, Hinterleitner et al. 1997, Duval-Iflah et al. 1999, Primi et al. 1999, Matheson and Noble 2000, Singh and Narayan 2008, Muheet et al. 2018). While these enzymes have potent antisecretory activity, they have little effect on gastrointestinal motility (Rachmilewitz et al. 1983, Schwartz 2000). Former studies on humans and animals reported that the drug is more effective and safer than other drugs (loperamide, atropine, diphenoxylate) and probiotics used to treat diarrhea (Bergmann et al. 1992, Fischbach et al. 2016). In human medicine, racecadotril is used without a prescription in many countries (CVMP 2020). *In vitro* and *in vivo*, racecadotril rapidly metabolizes to the pharmacologically more potent thiorphan. Racecadotril has a wide therapeutic window and its interactions with other drugs have not been reported (Bergmann et al. 1992, Lecomte 2000, World Health Organization 2007, Eberlin et al. 2012).

The aim of this study was to determine the clinical efficacy of racecadotril in the treatment of infectious diarrhea caused by *E. coli, Rotavirus, Coronavirus* and *C. parvum* in neonatal calves. This study is the first clinical study of the effect of racecadotril on calf diarrhea.

Materials and Methods

Animals

The study was carried out at Selcuk University, Faculty of Veterinary Medicine, Department of Internal Medicine, Türkiye. The Ethics Committee of Selcuk University, Faculty of Veterinary Medicine (no. 2017/28) approved the use of the animals for this study and all study protocols. The experimental group of the study consisted of 46 calves with diarrhea, 2-20 days old and 40-45 kg of weight in different breeds (29 Holstein and 17 Simmental) brought to the Selcuk University, Faculty of Veterinary Medicine, Internal Medicine Department with the complaint of diarrhea. All of the calves in the experimental group were brought within 6-24 hours after the onset of diarrhea and had not received any treatment. Fourteen healthy Holstein calves, 2-20 days old and weighing 40-45 kg, obtained from the faculty farm, formed the control group. The healthy calves without diarrhea or disease symptoms, hematological results within reference limits, and negative feces fast antigen testing (Rapid BoviD-5 Ag Test Kit (RC13-02DD), BioNote Inc, Gyeonggi-do, Korea) results formed the control group. All calves (experimental and control groups) included in the study were hospitalized for 72 hours in the hospitalization unit of the Internal Medicine Department. During the study the calves were housed individually. All calves were fed 10% of their body weight in fresh milk twice daily (Amaral-Phillips et al. 2006). Fresh milk was given by stomach tube to calves with diarrhea and no/weak suckling reflex. Healthy calves and calves with a suckling reflex during the treatment period were bottle fed. Healthy calves forming the control group and calves with diarrhea whose sucking reflex recovered during the treatment process were bottle fed.

Table 1. Clinical feces and dehydration scoring of calves with diarrhea.

Point	Dehydration degree (%)	CRT (s)	Mucose membran	Appetite	Mental status	Standing	Feces quality
3	Normal	1-2	Normal	Normal	Normal	Normal	Normal
2	5-8	3-4	Hyperemic	Slightly reluctant	Slight reduction in reflexes	Wobbly gait Can only stand up with help	Slightly soft, pastry consistency
1	9-12	4-6	Slightly cyanotic, slightly cold	Suction is weak	Mild loss of consciousness	Unable to straighten head forward Unable to stand up	Slightly watery
0	>12	>6	Cyanotic, cold, anemic	No sucking reflex	Complete loss of consciousness Shock Death	Lying in the lateral position Shock	Severe diarrhea, bloody-smelly

CRT – capillary refill time.

Clinical examinations

The breed, age and sex of all calves included in the study were recorded. Routine clinical examinations of all calves were performed before enrollment in the study. Body temperature and respiratory rate were also recorded. A modified scoring system was used by standardizing clinical examination findings and scoring each of the clinical parameters between 0 and 3 according to the degree of importance. Feces quality was evaluated by modifying the fecal consistency scoring developed by McGuirk (2008) and Renaud et al. (2020). For this purpose, a scoring system based on seven clinical parameters including dehydration degree, capillary refill time, mucous membranes, appetite, mental status, standing, and feces quality was used (Table 1). In order to evaluate the dehydration status of calves with diarrhea, the position of the eyeballs in the orbit was measured with a millimetric caliper. The capillary filling time of calves with diarrhea was evaluated as the time it took for blood to re-blood after pressing the mucous membrane with nails (Trefz et al. 2017).

Fecal samples analysis

Fast antigen testing (Rapid BoviD-5 Ag Test Kit (RC13-02DD), BioNote Inc, Gyeonggi-do, Korea) was used to detect the presence of bovine *Rota-* and *Coronavirus*, *K99 E. coli, Cryptosporidium,* and *Giardia* antigens in calves with diarrhea. The sensitivity and specificity of the fast antigen testing for infectious agents were 99% and 98% for *Rotavirus*, 98.4% and 98% for *Coronavirus*, 97.8 % and 99 % for *K99 E. coli*, 98.2% and 99% for *Cryptosporidium*, 92.1% and 99.1% for *Giardia*, respectively. The same test was applied to the control group. Those with negative test results were included in the control group. Additionally, diarrheal fecal samples with positive results on the fast antigen test for the presence of *C. parvum* were also examined by light microscopy for confirmation of the diagnosis.

Design of experimental and control groups

The calves included in the study were divided into 8 groups according to health status and the agents determined in the fast antigen test (Rapid BoviD-5 Ag Test Kit (RC13-02DD), BioNote Inc, Gyeonggi-do, Korea). Calves with diarrhea in which > 1 infectious agent was identified on fast antigen testing of feces were excluded from the study. During the study period (2 years), the number of animals in the experimental groups could not be equalized because a sufficient number of patients could not be obtained.

Healthy calves (HG)

This group consisted of 7 calves (n=7) who were determined to be healthy on the basis of clinical and laboratory examinations (blood gases, hemogram, fast antigen test, and microscopic examinations) and the scoring system. No treatment was applied to the calves.

Healthy calves administered racecadotril (HRG)

This group consisted of 7 calves (n=7) who were determined to be healthy on the basis of clinical and laboratory examinations (blood gases, hemogram, fast antigen test, and microscopic examinations) and the scoring system. Racecadotril was administered to the calves in this group.

Calves with *E. coli*-associated diarrhea (ECG)

This group consisted of 10 calves (n=10) with diarrhea detected as *E. coli* infection according to the fast

antigen test. The standard treatment protocol was applied to the calves in this group.

Calves with *E. coli*-associated diarrhea administered racecadotril (ECRG)

This group consisted of 8 calves (n=8) with diarrhea detected as *E. coli* infection according to the fast antigen test. Calves in this group received racecadotril and the standard treatment protocol.

Calves with bovine *Rotavirus/Coronavirus*-associated diarrhea (VG)

This group consisted of 7 calves (n=7) with diarrhea detected as *Rotavirus/Coronavirus* infection according to the fast antigen test. The standard treatment protocol was applied to the calves in this group.

Calves with bovine *Rotavirus/Coronavirus*-associated diarrhea administered racecadotril (VRG)

This group consisted of 8 calves (n=8) with diarrhea detected as *Rotavirus/Coronavirus* infection according to the fast antigen test. Racecadotril and standard treatment protocol were administered to the calves in this group.

Calves with *C. parvum*-associated diarrhea (CG)

This group consisted of 6 calves (n=6) with diarrhea detected as *C. parvum* infection according to fast antigen test and microscopic examinations. The standard treatment protocol was applied to the calves in this group.

Calves with *C. parvum*-associated diarrhea administered racecadotril (CRG)

This group consisted of 7 calves (n=7) with diarrhea detected as *C. parvum* infection according to the fast antigen test and microscopic examinations. Racecadotril and standard treatment protocol were administered to the calves in this group.

Racecadotril (Raxerin®, 30 mg, oral tablet, Ilco Pharmaceuticals, Konya, Türkiye) was administered orally at a dose of 2.5 mg/kg at 12 hour intervals for 3 days through a stomach tube. The first administration of racecadotril was at the time of admission.

Blood sample collection and analysis

Blood samples from all calves were taken from the jugular vein at the onset of treatment (0^{th} hour) and then 24 and 72 hours later. Blood was collected into heparinized syringes for blood gas measurements, and into evacuated blood collection tubes coated on the interior with spray dried K_3EDTA for the hemogram. Blood gas and electrolyte analysis included power of hydrogen (pH), partial pressure of carbon dioxide (pCO_2), bicarbonate (HCO_3), base excess (BE), potassium (K), sodium (Na), and lactate (Lac) and were performed using a blood gas analyzer (ABL 90 Flex, Radiometer, Brea, CA, USA). Hemogram parameters including total leukocytes (WBC) and hematocrit (Hct) were assessed using an automated hematologic analyzer (MS4e, Melet Schlosing Laboratories, Osny, France), within 5 to 10 minutes after blood samples were obtained.

Standard treatment protocol for diarrhea calves

In addition to fluid therapy, antimicrobials, hyperimmune serum, and supportive therapy were administered to calves with diarrhea as standard treatment. According to the blood gas analysis, isotonic (1.3%) sodium bicarbonate ($NaHCO_3$) (Carbotek®, Teknovet) with 5% dextrose (Dextrosol®, Vilsan) was administered to calves with diarrhea. Calves with diarrhea in need received the colloid fluid of 6% hydroxyethyl starch (Voluven®, Fresenius Kabi) in 0.9% NaCl solution (10 mL/kg/h). Calves with K99 *E. coli* diarrhea and *Cryptosporidium* infections received hyperimmune serum (15 mL, SC, once, Septicol®, Vetal) and halofuginone (0.1 mg/kg, PO, q 24 h for 7 days, Halocur®, MSD), respectively. Ceftiofur (2.2 mg/kg, IM, q 24 h for 5 days, Exenell®, Zoetis) was administered to calves with diarrhea. Calves with diarrhea received supportive treatment including a vitamin A, D_3, and E combination (1 mL, vitamin A, 500.000 U; vitamin D_3, 75.000 U and vitamin E, 50 mg, IM once, Ademin®, DİF) and vitamin C (3 mL, SC, q 24 h for 3 days, Vita-C Vetoquinol®, Novakim).

Statistical analysis

The statistical package program SPSS 25 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) was used to evaluate the data. The Kolmogorov-Smirnov test was used to determine the normality of variables and the homogeneity of variances. Parametric data (blood gas and hemogram parameters) were expressed as mean ± SD and were evaluated using one-way analysis of variance (ANOVA) and the post hoc Tukey test. Nonparametric data (clinical score parameters) were expressed as median (min-max) and were evaluated using the Kruskal-Wallis test and the post hoc Mann Whitney U test. A value of $p<0.05$ was accepted for the significance level of the tests.

Table 2. Effect of racecadotril on clinical score parameters in calves with diarrhea caused by *Escherichia coli*.

Parameters	HG			HRG			ECG			ECRG		
	0th h	24th h	72nd h	0th h	24th h	72nd h	0th h	24th h	72nd h	0th h	24th h	72nd h
Degree of dehydration (%)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	2^{b} (1-2)	2^{b} (2-3)	3^{a} (3-3)	1.5^{b} (0-3)	3^{ab} (2-3)	3^{a} (2-3)
CRT (s)	3^{ac} (3-3)	3^{ac} (3-3)	3^{ac} (3-3)	3^{a} (3-3)	3^{ac} (3-3)	3^{ac} (3-3)	1^{b} (0-2)	2^{bc} (0-3)	3^{ac} (2-3)	1^{b} (1-2)	2^{bc} (1-3)	3^{ac} (1-3)
Mucose	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	1^{b} (1-3)	1.5^{ab} (1-3)	3^{a} (2-3)	1^{b} (0-3)	2^{ab} (1-3)	3^{a} (2-3)
Appetite	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	0.5^{b} (0-1)	2^{ab} (1-3)	3^{a} (2-3)	0.5^{b} (0-1)	2^{ab} (1-3)	3^{a} (3-3)
Mental status	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	2^{bc} (1-2)	3^{c} (1-3)	3^{ac} (2-3)	1^{b} (0-2)	2.5^{abc} (1-3)	3^{ac} (3-3)
Standing	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	2^{b} (0-3)	3^{ab} (2-3)	3^{a} (3-3)	1.5^{b} (0-3)	3^{ab} (2-3)	3^{a} (3-3)
Feces quality	3^{a} (3-3)	3^{a} (2-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (2-3)	3^{a} (3-3)	0.5^{b} (0-2)	1^{b} (0-1)	2.5^{ab} (2-3)	0^{b} (0-2)	1^{ab} (0-3)	3^{a} (2-3)
Body temperature °C	37.9 (37.5-39.0)	38.3 (37.8-39)	38.5 (37.8-39.0)	38.5 (37.9-38.9)	38.5 (38.2-38.8)	38.3 (37.9-38.5)	38.5 (36-39.2)	38.5 (37.9-39.2)	38.4 (38-39.2)	38.3 (33.0-39.0)	38.7 (38.2-39.4)	38.5 (38-38.8)

Data are presented as Median (min-max).

Different letters (a, b, c) in the same line indicate statistically (ANOVA, posthoc Tukey; $p<0.05$) significant difference between groups. HG – healthy control group, HRG – healthy control + racecadotril administered group, ECG – *Escherichia coli* infected group, ECRG – *Escherichia coli* infected+racecadotril administered group, CRT – capillary refill time.

Results

Clinical scoring findings

According to the feces and dehydration scoring system, all parameters except body temperature were statistically different ($p<0.05$) in calves on ECG (Table 2). After the treatment, the degree of dehydration, mental status, and feces consistency improved after 24 hours in the ECRG and after 72 hours in the ECG. It was observed that racecadotril contributed significantly to clinical improvement and solidification of feces consistency in calves with diarrhea caused by *E. coli*.

According to the feces and dehydration scoring system, all parameters except mucosa and body temperature were statistically different in *Cryptosporidium parvum* infected calves ($p<0.05$; Table 3). It was determined that the differences had improved at the 24th and 72nd hours (fecal quality) following the treatment and racecadotril did not contribute to the improvement of these parameters.

According to the feces and dehydration scoring system, all parameters were found to be statistically different ($p<0.05$) in the group of calves with bovine *Rotavirus*/*Coronavirus*- associated diarrhea, except for body temperature (Table 4). It was determined that all parameters had improved at the 24th hour following the treatment and racecadotril did not contribute to the healing process, except for feces quality. The clinical score parameters in ECG, CPG, and VG were found to be similar, except for body temperature. Of the 46 diarrheal calves included in the study, 42 responded to treatment, while four died. Of the four calves that died, two were in CPG, 1 in VG, and 1 in VRG.

Lethargy, insufficiency in the sucking reflex, dehydration, difficulty in standing or maintaining sternal recumbency, watery feces, decreased interest in the environment, and hypothermia were observed in all of the calves with diarrhea. After 24 hours of treatment, no signs of depression, dehydration, or weakness/difficulty standing up were observed in calves with diarrhea, while sucking reflexes and appetites were observed to have increased significantly. After 24 hours of treatment, the feces consistency was slightly solidified, but the feces consistency did not reach normality. After the 48th hour of treatment, it was determined that most of the calves with diarrhea were standing and seemed environmentally aware. The feces consistency continued to solidify but did not reach normal levels. At the end of 72 hours of treatment, 21 of 46 calves with diarrhea had improved feces quality and scored 3 points according to feces scoring (Table 1). When the fecal quality of the calves was compared at the end of the treatment, it was observed that the fecal quality of 5 calves in the ECG, 1 in the ECRG, 3 in the CPG, 4 in the CPRG, 7 in the VG, and 5 in the VRG were slightly soft (2 points).

Blood gas and hemogram findings

Racecadotril did not cause any changes in blood gas and hemogram parameters in healthy calves. Blood pH,

Table 3. Effect of racecadotril on clinical score parameters in calves with diarrhea caused by *Cryptosporidium parvum.*

Parameters	HG			HRG			CPG			CPRG		
	0th h	24th h	72nd h	0th h	24th h	72nd h	0th h	24th h	72nd h	0th h	24th h	72nd h
Degree of dehydration (%)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	1.5^{b} (1-2)	3^{ab} (2-3)	3^{ab} (2-3)	1^{b} (1-3)	2^{ab} (2-3)	3^{a} (2-3)
CRT (s)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	1.5^{b} (1-2)	2^{ab} (1-3)	2^{ab} (0-3)	1^{b} (1-3)	1^{b} (1-2)	3^{ab} (2-3)
Mucose	3 (3-3)	3 (3-3)	3 (3-3)	3 (3-3)	3 (3-3)	3 (3-3)	1 (1-3)	1.5 (1-3)	2 (1-3)	1 (1-3)	2 (1-3)	3 (1-3)
Appetite	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	1^{b} (0-2)	2.5^{ab} (0-3)	3^{ab} (0-3)	0^{b} (0-3)	2^{ab} (1-3)	3^{ab} (1-3)
Mental status	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	2^{ab} (0-3)	2^{ab} (0-3)	3^{ab} (0-3)	2^{b} (1-3)	3^{ab} (1-3)	3^{ab} (2-3)
Standing	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	1.5^{ab} (0-3)	2^{ab} (0-3)	3^{ab} (1-3)	1^{b} (0-3)	3^{ab} (1-3)	3^{a} (3-3)
Feces quality	3^{a} (3-3)	3^{ac} (2-3)	3^{a} (3-3)	3^{a} (3-3)	3^{ac} (2-3)	3^{a} (3-3)	0^{b} (0-1)	1^{b} (0-3)	3^{ab} (1-3)	1^{bc} (0-2)	1^{b} (0-2)	2^{ab} (1-3)
Body temperature °C	37.9 (37.5-39.0)	38.3 (37.8-39)	38.5 (37.8-39.0)	38.5 (37.9-38.9)	38.5 (38.2-38.8)	38.3 (37.9-38.5)	38.2 (37.3-39.7)	38.1 (37.1-38.4)	38 (35.5-39)	37.3 (33.2-39.1)	38.2 (37.8-39.4)	38.1 (38.0-39.0)

Data are presented as Median (min-max).
Different letters (a, b, c) in the same line indicate statistically (Kruskal-Wallis; $p<0.05$) significant difference between the groups. HG – healthy control group, HRG – healthy control + racecadotril administered group, CPG – *Cryptosporidium parvum* infected group (CPG), CPRG – *Cryptosporidium parvum* infected + racecadotril administered group, CRT – capillary refill time.

Table 4. Effect of racecadotril on clinical score parameters in calves with diarrhea caused by *Rota/Coranavirus*.

Parameters	HG			HRG			VG			VRG		
	0th h	24th h	72nd h	0th h	24th h	72nd h	0th h	24th h	72nd h	0th h	24th h	72nd h
Degree of dehydration (%)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	1^{b} (1-2)	2^{ab} (2-3)	3^{ab} (2-3)	1^{b} (1-2)	2^{ab} (1-3)	3^{ab} (1-3)
CRT (s)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	1.5^{b} (1-2)	2^{ab} (1-3)	3^{ab} (1-3)	1^{b} (1-2)	2^{ab} (1-2)	2.5^{ab} (1-3)
Mucose	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	1^{b} (1-3)	1.5^{ab} (1-3)	3^{ab} (1-3)	1^{b} (0-1)	2^{ab} (0-3)	2.5^{ab} (0-3)
Appetite	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	0^{b} (0-2)	2.5^{ab} (1-3)	3^{a} (1-3)	0^{b} (0-1)	3^{a} (1-3)	3^{a} (2-3)
Mental status	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	2^{b} (1-3)	3^{ab} (2-3)	3^{a} (2-3)	1^{b} (1-2)	3^{ab} (2-3)	3^{a} (0-3)
Standing	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (3-3)	1^{ab} (0-3)	3^{ab} (2-3)	3^{ab} (3-3)	1^{b} (1-3)	3^{ab} (2-3)	3^{ab} (2-3)
Feces quality	3^{a} (3-3)	3^{a} (2-3)	3^{a} (3-3)	3^{a} (3-3)	3^{a} (2-3)	3^{a} (3-3)	0^{b} (0-1)	1^{b} (0-2)	2^{ab} (2-3)	0^{b} (0-1)	1^{b} (0-2)	2^{ab} (1-3)
Body temperature °C	37.9 (37.5-39.0)	38.3 (37.8-39)	38.5 (37.8-39.0)	38.5 (37.9-38.9)	38.5 (38.2-38.8)	38.3 (37.9-38.5)	36.2 (33.2-38.7)	38.3 (37.3-38.9)	38 (35.5-38.4)	38.1 (36.3-39.4)	38.8 (37.0-39.2)	38.5 (37.5-39.6)

Data are presented as Median (min-max).
Different letters (a, b, c) in the same line indicate statistically (Kruskal-Wallis; $p<0.05$) significant difference between the groups. HG – healthy control group, HRG – healthy control + racecadotril administered group, VG – *Rotavirus* and/or *Coronavirus* infected, VRG – *Rotavirus/Coronavirus* infected+ racecadotril administered group, CRT – capillary refill time.

HCO_3, BE, Lac, and WBC values in ECG were found to be significant ($p<0.05$) compared to HG (Table 5). Blood pH, HCO_3, BE, K, sodium (Na), and WBC values were found to be statistically different ($p<0.05$) in CPG compared to HCG (Table 6). Blood pH, HCO_3, BE, K, Na, Lac, and WBC values were found to be statistically different ($p<0.05$) in VG compared to HCG (Table 7). It was determined that these differences disappeared 24 hours after the treatment. The common parameters varying in relation to HCG in calves in ECG, CPG, and VG groups were pH, HCO_3, and BE. While Lac changed in ECG and VG, the covariate parameter of CPG and VG was WBC.

Table 5. Effect of racecadotril on some blood gas and hemogram parameters in calves with diarrhea caused by *Escherichia coli.*

Parameter	HG			HRG			ECG			ECRG		
	0th h	24th h	72nd h	0th h	24th h	72nd h	0th h	24th h	72nd h	0th h	24th h	72nd h
pH	7.40±0.03^{a}	7.39±0.01^{a}	7.36±0.03^{a}	7.40±0.02^{a}	7.41±0.04^{a}	7.38±0.02^{a}	7.19±0.11^{bc}	7.31±0.11^{ab}	7.36±0.07^{a}	7.10±0.11^{c}	7.36±0.04^{a}	7.37±0.06^{a}
pCO_2 (mmHg)	44.9±1.64	45.5±2.26	45.1±2.17	44.5±5.82	39.8±3.44	45.1±3.52	45.8±11.5	47.3±5.20	46.4±7.87	44.3±14.2	46.8±6.98	48.4±7.30
HCO_3 (mol/L)	25.7±2.32^{a}	26.3±1.56^{a}	24.4±1.62^{a}	24.5±1.55^{a}	24.7±2.19^{a}	25.3±0.98^{a}	16.1±4.14^{b}	22.3±4.83^{a}	24.7±3.50^{a}	12.4±3.08 ^{b}	24.7±3.10^{a}	25.5±2.32^{a}
BE (mmol/L)	2.54±2.82^{a}	3.14±2.10^{a}	0.77±2.19^{a}	0.86±1.74^{a}	0.93±2.42^{a}	2.16±1.11^{a}	-10.31±6.55^{b}	-2.08±6.63^{a}	0.95±4.85^{a}	-15.9±5.12^{b}	1.23±3.83^{a}	2.78±2.34^{a}
K (mmol/L)	4.37±0.14	4.28±0.24	4.91±0.38	4.25±0.43	4.40±0.56	4.08±0.17	4.89±0.84	4.70±0.54	5.44±1.74	5.12±0.67	4.68±0.67	4.55±0.31
Na (mmol/L)	142±5.09	142.8±4.41	142.4±6.10	143.1±2.85	141.6±2.50	144.6±2.33	138.7±6.68	139.4±6.40	140.2±4.47	139.0±6.99	144.3±5.28	140.3±6.41
Lac (mmol/L)	1.51±0.67^{c}	1.05±0.35^{c}	1.65±0.63^{c}	1.15±0.74^{c}	0.45±0.08^{c}	1.78±2.09^{bc}	4.71±3.04^{ab}	2.28±1.31^{bc}	1.75±0.42^{bc}	7.07±3.63^{a}	2.27±1.14^{b}	2.05±0.92^{b}
WBC (m/mm^3)	9,67±1,29^{ab}	9,84±1,66^{ab}	8,42±2,25^{b}	11,5±6,39^{ab}	10,3±4,86^{ab}	8,63±2,73^{b}	12,3±4,44^{ab}	12,4±6,01^{ab}	10,1±2,90^{ab}	17,5±7,19^{a}	12,3±5,08^{ab}	11,2±3,50^{ab}
HTC (%)	29.05±5.99	29.2±5.23	29.8±4.40	28.8±7.54	27.9±6.93	28.8±7.41	36.7±8.10	32.6±6.53	31.4±7.39	39.1±8.48	33.9±8.39	31.5±6.67

Data are presented as mean±SD.Different letters (a, b, c) in the same line indicate statistically (ANOVA, posthoc Tukey; $p<0.05$) significant difference between groups. HG – healthy control group, HRG – healthy control+ racecadotril administered group, ECG – *Escherichia coli* infected group, ECRG – *Escherichia coli* infected+ racecadotril administered group, pCO_2 – partial pressure of carbon dioxide, HCO_3 – bicarbonate, BE – base deficit, K – potassium, Na – sodium, Lac – lactate, WBC – total leukocytes, HTC – hematocrit.

Table 6. Effect of racecadotril on some blood gas and hemogram parameters in calves with diarrhea caused by *Cryptosporidium parvum.*

Parameter	HG			HRG			CPG			CPRG		
	0th h	24th h	72nd h	0th h	24th h	72nd h	0th h	24th h	72nd h	0th h	24th h	72nd h
pH	7.40±0.03^{ab}	7.39±0.01^{ab}	7.36±0.03 ^{ab}	7.40±0.02^{ab}	7.41±0.04^{a}	7.38±0.02^{ab}	7.15±0.08^{d}	7.29±0.10^{bc}	7.37±0.06^{ab}	7.20±0.11^{cd}	7.31±0.04^{abc}	7.38±0.10^{ab}
pCO_2 (mmHg)	44.9±1.64^{ab}	45.5±2.26^{ab}	45.1±2.17^{ab}	44.5±5.82^{ab}	39.8±3.44^{ab}	45.1±3.52^{ab}	37.1±5.68^{b}	45.9±7.09^{ab}	51.3±10.6^{a}	37.4±6.32^{b}	47.3±2.70^{ab}	48.4±14.2^{ab}
HCO_3 (mmol/L)	25.7±2.32^{ab}	26.3±1.56^{ab}	24.4±1.62^{ab}	24.5±1.55^{ab}	24.7±2.19^{ab}	25.3±0.98^{ab}	12.8±3.37^{c}	20.9±5.65^{b}	27.0±3.83^{a}	14.5±3.42^{c}	21.9±2.54^{ab}	25.8±2.48^{ab}
BE (mmol/L)	2.54±2.82^{ab}	3.14±2.10^{ab}	0.77±2.19^{ab}	0.86±1.74^{ab}	0.93±2.42^{ab}	2.16±1.11^{ab}	-15.6±5.66^{c}	-4.05±7.58^{b}	4.24±4.99^{a}	-13.2±5.35^{c}	-2.07±3.22^{ab}	2.64±2.74^{ab}
K (mmol/L)	4.37±0.14^{abc}	4.28±0.24^{abc}	4.91±0.38^{abc}	4.25±0.43^{bc}	4.40±0.56^{abc}	4.08±0.17^{bc}	5.68±1.55^{a}	4.10±1.18^{bc}	3.78±0.38^{c}	5.33±1.09^{ab}	4.34±0.72^{abc}	4.43±0.52^{abc}
Na (mmol/L)	142±5.09^{ab}	142.8±4.41^{ab}	142.4±6.10^{ab}	143.1±2.85^{ab}	141.6±2.50^{ab}	144.6±2.33^{ab}	143.6±18.1^{ab}	154.8±15.7^{a}	149.4±14.7^{a}	127.4±5.62^{b}	140.5±9.80^{ab}	138.2±8.86^{ab}
Lac (mmol/L)	1.51±0.67	1.05±0.35	1.65±0.63	1.15±0.74	0.45±0.08	1.78±2.09	3.38±2.31	3.35±2.42	3.18±2.52	3.57±3.71	1.63±0.67	1.27±1.39
WBC (m/mm^3)	9.67±1.29^{d}	9.84±1.66^{cd}	8.42±2.25^{d}	11.5±6.39^{bcd}	10.3±4.86^{cd}	8.63±2.73^{d}	25.5±9.90^{a}	22.5±9.98^{abc}	17.9±8.16^{abcd}	24.2±7.65^{ab}	17.3±10.9^{abcd}	16.9±5.96^{abcd}
HTC (%)	29.05±5.99	29.2±5.23	29.8±4.40	28.8±7.54	27.9±6.93	28.8±7.41	38.7±12.2	35.0±11.4	35.4±13.12	40.8±8.91	37.5±6.83	35.8±9.67

Data are presented as mean±SD.

Different letters (a, b, c) in the same line indicate statistically (ANOVA, posthoc Tukey; $p<0.05$) significant difference between groups. HG – healthy control group, HRG – healthy control + racecadotril administered group, CPG – *Cryptosporidium parvum* infected group (CPG), CPRG – *Cryptosporidium parvum* infected + racecadotril administered group, pCO_2 – partial pressure of carbon dioxide, HCO_3 – bicarbonate, BE – base deficit, K – potassium, Na – sodium, Lac – lactate, WBC – total leukocytes, HTC –hematocrit.

Discussion

Diarrhea is the most serious disease encountered in newborn calves (Ok et al. 2009, Sen et al. 2009, Coskun et al. 2010, Trefz et al. 2015, Ok et al. 2020). Enterotoxigenic *E. coli* is the most common bacterial cause of diarrhea in newborn calves. *E. coli* serotypes (K99, K101, F41) with adhesion factor plus antigen cause secretory diarrhea by releasing enterotoxin. The most common among these is enterotoxigenic *E. coli* with K99 pilus (Ok et al. 2009, Gibbons et al. 2014, Ok et al. 2020). Generally, after 5 days of age, *Coronavirus*, *Rotavirus,* and *C. parvum* causes diarrhea in neonatal calves. *Rotavirus* and *Coronavirus* cause osmotic diarrhea by attaching to the villous epithelial cells of the small intestine and epithelial cells on the crypt surfaces of the colonic mucosa, causing villous loss. In particular, *Coronavirus* does not only cause severe diarrhea and dehydration due to villous damage but also creates an environment for the reproduction of *E. coli*. This situation leads to systemic infection in severe cases, increasing the risk of death significantly (Tsunemitsu et al. 1999, Ok et al. 2009, Ok et al. 2020). Another important infectious agent causing diarrhea in neonatal calves is *C. parvum* (Björkman et al. 2003, Ok et al. 2009, Ok et al. 2021).

Table 7. Effect of racecadotril on some blood gas and hemogram parameters in calves with diarrhea caused by *Rota/Coronavirus*.

Parameter	HG			HRG			VG			VRG		
	0th h	24th h	72nd h	0th h	24th h	72nd h	0th h	24th h	72nd h	0th h	24th h	72nd h
pH	7.40±0.03^{a}	7.39±0.01^{a}	7.36±0.03^{a}	7.40±0.02^{a}	7.41±0.04^{a}	7.38±0.02^{a}	7.03±0.13^{b}	7.32±0.09^{a}	7.29±0.09**a**	7.14±0.11^{b}	7.31±0.05^{a}	7.38±0.05^{a}
pCO_2 (mmHg)	44.9±1.64	45.5±2.26	45.1±2.17	44.5±5.82	39.8±3.44	45.1±3.52	51.7±14.4	47.1±6.32	50.5±4.61	40.8±7.78	45.1±4.61	47.1±7.11
HCO_3 (mmol/L)	25.7±2.32^{a}	26.3±1.56^{a}	24.4±1.62^{a}	24.5±1.55^{a}	24.7±2.19^{a}	25.3±0.98^{a}	12.2±3.77^{b}	22.7±4.61^{a}	21.9±4.11^{a}	13.7±3.93^{b}	21.3±3.61^{a}	25.9±5.13^{a}
BE (mmol/L)	2.54±2.82^{a}	3.14±2.10^{a}	0.77±2.19^{a}	0.86±1.74^{a}	0.93±2.42^{a}	2.16±1.11^{a}	-16.4±7.03^{b}	-1.40±6.49^{a}	-2.14±5.44^{a}	-14.4±6.79^{b}	-3.04±4.84^{a}	2.42±6.41^{a}
K (mmol/L)	4.37±0.14^{b}	4.28±0.24^{b}	4.91±0.38^{ab}	4.25±0.43^{b}	4.40±0.56^{b}	4.08±0.17^{b}	6.66±1.61^{a}	4.74±1.13^{b}	5.27±0.91^{ab}	5.54±1.80^{ab}	4.64±1.31^{b}	4.47±1.00^{b}
Na (mmol/L)	142±5.09^{ab}	142.8±4.41^{ab}	142.4±6.10^{ab}	143.1±2.85^{ab}	141.6±2.50^{ab}	144.6±2.33^{ab}	128.1±17.2^{b}	143.3±20.0^{ab}	135.8±12.2^{ab}	137.4±10.6^{ab}	144.1±5.15^{ab}	147.8±8.04^{a}
Lac (mmol/L)	1.51±0.67^{b}	1.05±0.35^{b}	1.65±0.63 ^{b}	1.15±0.74^{b}	0.45±0.08^{b}	1.78±2.09^{b}	5.65±4.15^{a}	2.31±1.25^{b}	2.54±2.00^{ab}	2.79±2.45^{ab}	1.73±1.00^{b}	1.07±0.49^{b}
WBC (m/mm^3)	9,67±1,29^{b}	9,84±1,66^{b}	8,42±2,25^{b}	11,5±6,39^{b}	10,3±4,86^{b}	8,63±2,73^{b}	27,3±20,6^{a}	19,1±9,66^{ab}	14,3±4,02^{ab}	18,9±9,23^{ab}	11,7±3,58^{b}	10,9±3,51^{b}
HTC (%)	29.05±5.99	29.2±5.23	29.8±4.40	28.8±7.54	27.9±6.93	28.8±7.41	42.6±13.4	36.9±8.56	37.9±6.22	36.9±11.26	31.7±8.19	31.8±9.29

Data are presented as mean±SD.

Different letters (a, b, c) in the same line indicate statistically (ANOVA, posthoc Tukey; $p<0.05$) significant difference between groups. HG – healthy control group, HRG – healthy control + racecadotril administered group, VG – *Rotavirus* and/or *Coronavirus* infected, VRG – *Rotavirus/Coronavirus* infected+ racecadotril administered group, pCO_2 – partial pressure of carbon dioxide, HCO_3 – bicarbonate, BE – base deficit, K – potassium, Na – sodium, Lac – lactate, WBC – total leukocytes, HTC – hematocrit.

As reported by previous researchers (Tsunemitsu et al. 1999, Ok et al. 2009, Gibbons et al. 2014, Al Mawly et al. 2015, Ok et al. 2020, Ok et al. 2021), we found that the causative agent was *E. coli* (K99) in 2-4 days old diarrheal calves and *Rotavirus, Coronavirus,* and *C. parvum* were the agents in 5-20 days old diarrheal calves. This is the first study to evaluate the clinical efficacy of racecadotril in the treatment of neonatal calf diarrhea. In the clinical evaluation of recovery, we observed that 42 of the 46 treated calves recovered and 4 died. Of the dead calves, 2 were in CPG, 1 in VG, and 1 in VRG. When the calves in the racecadotril-treated and non-administered groups were compared in relation to feces scoring it was observed that although 1 of 8 calves in ECRG treated with racecadotril, 4 of 7 calves in CPRG, and 5 of 7 calves in VRG improved clinically, feces consistency did not improve within 72 hours (feces score 2, slightly soft). Although 5 of 10 calves in ECG, 3 of 6 calves on CPG, and 7 of 8 calves on VG improved clinically, feces consistency did not fully return to normal (feces score 2, slightly soft) within 72 hours. Both clinical improvement and feces full solidification (feces score point 3, complete solidification) of 7 of 8 calves on ECRG within 72 hours showed that racecadotril was effective in reducing intestinal secretion in secretory diarrhea caused by *E. coli* toxins. Our view is consistent with the view that racecadotril reduces diarrhea caused by cholera toxin in humans and dogs by preventing fluid secretion in the jejunum (Hinterleitner et al. 1997, Primi et al. 1999). In addition, Muheet et al. (2018) reported that the administration of racecadotril at a dose of 1 mg/kg 3 times a day to dogs with diarrhea improved feces consistency and shortened the duration of diarrhea. We observed that racecadotril administered at a dose of 2.5 mg/kg twice a day for 3 days in *E. coli* diarrheal calves improved feces consistency and shortened the duration of diarrhea. On the other hand, it has been observed that racecadotril administration is not effective in the treatment of neonatal calf diarrhea caused by *Coronavirus, Rotavirus,* and *C. parvum.* The reason why racecadotril is not effective in these cases may be related to the fact that viruses and protozoa cause osmotic diarrhea by causing damage to the cryptic villi and such villi need a long time to regenerate. In addition, it was observed that the administration of racecadotril to healthy calves did not have any side effects. In conclusion, it can be said that the addition of racecadotril to the standard treatment of *E. coli*-induced calf diarrhea is quite beneficial (Table 2, Table 5). The beneficial effect of racecadotril may be related to its antisecretory effect in the intestines and this effect can reduce fluid-electrolyte losses, which are serious in young animals.

Metabolic acidosis occurs as a result of extracellular electrolyte (Na, K, and chlorine) and bicarbonate loss in calves with diarrhea caused by *E. coli*, *Rotavirus*, *Coronavirus,* and *C. parvum* (Sen et al. 2009, Constable et al. 2016, Ok et al. 2020). In most cases, plasma Na^+ concentration is moderately decreased but sometimes remains at normal levels. Plasma K^+ concentration is mainly affected by metabolic acidosis and disruption of the Na^+/K^+-ATPase pump and is generally increased (Sen et al. 2009, Constable et al. 2016). Ok et al. (2020) determined a decrease in pH, partial pressure of carbon dioxide (pCO_2), partial pressure of oxygen (pO_2), saturation of oxygen ($SatO_2$), HCO_3, and BE levels and an increase in Lac and K levels in venous blood gas in calves with diarrhea caused by *E. coli*, *Rotavirus, Coronavirus,* and *Cryptosporidium* agents. In the

present study, a statistically significant decrease in pH, HCO_3, and BE levels in the venous blood gas of the calves in the experimental groups was noted while an increase in lactate was determined, compared to the control group. In addition, a statistically significant difference was observed at 0.th of K and Na concentrations of viral (*Rota/Coronavirus*) and *Cryptosporidium parvum*-infected calves compared to post-treatment time points. These findings showed that metabolic acidosis developed as a result of gastrointestinal fluid-electrolyte losses in calves with diarrhea (Table 5, Table 6, Table 7) (Sen et al. 2009, Constable et al. 2016, Ok et al. 2020).

The way to prevent death in calves with diarrhea is to apply appropriate intravenous or oral fluid therapy and also chemotherapeutics for infectious agents (Berchtold 2009). The purposes of the use of these fluids are to restore fluid and electrolytes lost with feces, normalize circulation, and reduce multi-organ dysfunction by increasing the oxygen transport capacity to tissues (Berchtold 2009, Smith and Berchtold 2014). Fluids used in calves with diarrhea should be chosen depending on clinical findings, degree of dehydration, and acid-base imbalance (Berchtold 2009, Şen et al. 2013, Ok et al. 2020). Dehydration and metabolic acidosis caused by diarrhea should be corrected as early as possible. Alkaline agents are used for this purpose. The most important solution that can be given in severe acidemia is isotonic sodium bicarbonate (1.3% $NaHCO_3$). Acetate and lactated polyionic solutions can be used in mild acidemia cases (Berchtold 2009, Smith and Berchtold 2014, Trefz et al. 2017).

In the present study, it was observed that crystalloid (1.3% $NaHCO_3$) and 5% dextrose solutions applied in 42 of 46 calves with diarrhea quickly corrected acidemia and caused a marked clinical improvement within 24-48 hours. It was observed that 4 of the calves with diarrhea (2 in CPG, 2 in VG) did not respond to the treatment and died. The effective treatment of calf diarrhea is based on replacing the lost fluid electrolytes and reducing the loss, since the cause of death is acidemia due to the loss of fluid-electrolytes. As reported by many researchers (Berchtold 2009, Şen et al. 2013, Smith and Berchtold 2014, Trefz et al. 2017, Tsukano et al. 2017, Aydogdu et al. 2018, Ok et al. 2020) for the treatment of diarrhea-induced acidemia in calves, the application of 5% dextrose solution with 1.3 % of $NaHCO_3$ was effective in this study. The cornerstone of diarrhea treatment is fluid therapy to meet fluid, acid-base, and electrolyte deficiencies and to provide nutritional support (Smith and Berchtold 2014). Regardless of the etiology, calves with diarrhea often have increased coliform bacterial numbers in the small intestine; small intestinal bacterial overgrowth is associated with altered small intestinal function, morphologic damage, and increased susceptibility to bacteremia and endotoxemia. Therefore, parenteral administration of antimicrobials with a predominantly Gram-negative spectrum of activity is recommended (Constable 2009). Essential oils, probiotics, prebiotics, and yeast preparations are also used as potential supportive treatments in calves with diarrhea. It has been reported that these practices reduce the severity and duration of diarrhea and that more research is needed to reveal their clinical and economic importance (Katsoulos et al. 2017, Renaud et al. 2019). In this study, antibiotics and supportive treatments in addition to fluid therapy improved both clinical and metabolic status within 72 hours. Consistent with previous research results (Constable 2009, Smith and Berchtold 2014, Katsoulos et al. 2017, Renaud et al. 2019), we observed that antibiotics and supportive treatments in addition to fluid therapy improved both the clinical picture and the metabolic picture within 72 hours. In this study, racecadotril, which has a reducing effect on intestinal fluid secretion, was used for the first time in calves with diarrhea and it has been found to be effective in calf diarrhea caused by *E. coli* (Table 5). When the effect of racecadotril on clinical score parameters was evaluated, it was observed that the degree of dehydration, CRT, mucosa, appetite, mental status, and standing parameters returned to normal within 72 hours in calves with diarrhea in the experimental groups (Score 3). When the effect of racecadotril on feces quality was evaluated, it was determined that the score was 2 in the CPRG and VRG and 3 in the ECRG within 72 hours (Table 2, Table 3, Table 4). It can be said that racecadotril is very effective in improving both the clinical picture and feces quality in calves with *E. coli* diarrhea (Table 2).

The number of leukocytes increases in all calves with diarrhea caused by *E. coli* and *Cryptosporidium* species (Kumar et al. 2010, Malik et al. 2013, Megeed et al. 2015, Aydogdu et al. 2018, Ok et al. 2020). The studies on the change of leukocytes in calves with diarrhea caused by *Rotavirus* and *Coronavirus* are very limited. Ok et al. (2020) determined an increase in leukocyte counts in calves with diarrhea caused by *Rotavirus* and *Coronavirus*. In the present study, a statistically significant increase was found in WBC levels in all diarrheal calves compared to the control group.

Racecadotril can be used as a supportive treatment in addition to the etiological treatment of diarrhea due to bacterial infections. It may also be useful in non-infectious diarrhea where the integrity of the small intestine is not impaired.

According to the results of the study, i) racecadotril

is useful in the treatment of bacterial diarrhea, and ii) studies should be conducted to determine the clinical effectiveness of racecadotril in diarrhea caused by different bacteria species and dosage regimens of its in calves.

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