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The determination of treatment effect of chitosan oligosaccharide in lambs with experimentally cryptosporidiosis

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ABSTRACT

In this study, it was aimed to investigate the efficacy of chitosan oligosaccharide administrations in different doses of experimental infected lambs with *Cryptosporidium parvum*. 32 male lambs were used in the study and the lambs were divided into 4 groups with 8 lambs in each group. Groups 1, 2 and 3, twice a day, were administered chitosan oligosaccharide at a dose of 100, 500, and 1000 mg/kg for 7 days, respectively, with milk replacer. In group 4, lambs with cryptosporidiosis were subjected to normal feeding as control without drug administration. Clinical examinations of lambs were made before treatment (day 0) and on days 1, 3, 5 and 7 after treatment and 5 ml of blood was collected from vena jugularis for blood analysis of all lambs. Weight changes of lambs were recorded at 0, 7, 14, and 21 days. Stool specimens were collected pre-treatment (day 0) and on days 1, 3, 5, 7, 14 and 21 post-treatment to determine oocyst excretion of lambs with cryptosporidiosis. Lambs with a mean oocyte counts > 10 after stool examination were included to the treatment. Changes in clinical hematology, blood gases and biochemical parameters were observed during the course of treatment, but these changes were limited. Weight loss was observed at 7th day according to 0th day the lambs with experimental cryptosporidiosis but gradually weight increase was observed at 14th and 21st days and these changes were similar in all groups. Oocyst excretion decreased in all groups during treatment. According to 0th day, there was a significant ($p < 0.05$) decrease in oocyte excretions in the third day in group 1 and 2, and in day 5 in the group 3 and 4. Significant changes ($p < 0.05$) were observed in oocyst excretions on the third and fifth days among the groups. As a result, in lambs with experimental cryptosporidiosis, chitosan oligosaccharide improved in clinical signs and stool character shorter than the positive control group and the administration of chitosan oligosaccharide at doses of 100, 500 and 1000 mg/kg for 7 days significantly reduced oocyst excretion but not enough to remove cryptosporidiosis completely.

1. Introduction

Infection caused by *Cryptosporidium parvum* is common among young ruminants and is seen in various mammalians including humans. The disease has a high prevalence across the world and is among the important causes of diarrhea in neonatal farm animals (Scott, 2007; Paraud and Chartier, 2012; Constable et al., 2017). *C. parvum* is one of the primary causes of diarrhea seen in young lambs and goats. Diarrhea can develop as a result of *C. parvum* infection alone; however, it mostly develops due to mixed infection. Infection can occur as a severe diarrhea outbreak which can cause high mortality in lambs aged 4–10 days (Zorana et al., 2006; Goma et al., 2007; Paraud and Chartier, 2012; Constable et al., 2017). There is no specific treatment for cryptosporidiosis. It has been reported that paromomycin, lasalocid, halofuginone lactate, sulfoxinoxalin, azithromycin and toltrazuril have partial or demonstrable effect against in neonatal ruminants with cryptosporidiosis (Viu et al., 2000; Wright and Coop, 2007; Giadinis et al., 2008; Navarre et al., 2012; Yagci et al., 2017; Aydogdu et al., 2018).

Chitosan is a natural polysaccharide produced by deacetylation of chitin. It is non-toxic, biocompatible and biodegradable (Huang et al., 2004; Zhao et al., 2018). Chitin is the main component of crustaceans (crab, shrimp, etc.) and is also found in skeletons of insects and cell walls of fungi. There are many derivatives of chitin and the most important one is chitosan (Kumar, 2000). Chitosan and chitosan oligosaccharide (COS) have important uses in the medical field, such as controlled drug release, artificial blood vessels, antidiabetic, antibacterial, antifungal and hemostatic effect (Baldrick, 2010; Xia et al., 2011; Muanprasat and Chatsudthipong, 2017). In a study on the

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treatment of calves with diarrhea, oral chitosan oligosaccharide administration has been reported to be successful in treatment (Alam et al., 2012). In studies conducted, chitosan has been reported to inhibit the *in vitro* development of *C. parvum* (Luzardo Álvarez et al., 2012; Adjou et al., 2014; Mammeri et al., 2018). There is no study found in which the efficacy of chitosan oligosaccharide in the treatment of cryptosporidiosis in neonatal ruminants has been determined. According to this information, this study is the first to determine the efficacy of chitosan oligosaccharide in the treatment of cryptosporidiosis in lambs. Considering that chitosan inhibits the development of *C. parvum* in *in vitro* studies, we hypothesized that oral chitosan administration may be used in the treatment of lambs with experimentally cryptosporidiosis. The aim of this study was to determine the treatment efficacy of oral chitosan oligosaccharide in lambs with experimental cryptosporidiosis.

2. Materials and methods

2.1. Study animals

Ethics committee approval of the project was taken from Sivas Cumhuriyet University Animal Experiments Local Ethics Committee (31.08.2015/69). The study was conducted between 2016-2018. Experimental procedure was started simultaneously in all groups. In our study, a total of 32 male lambs younger than 5 days of age, 8 lambs in every 4 groups, were used. Stool specimens of lambs were collected and tested using rapid pen-side test (Bio-X Diagnostics S.A. Belgium) in terms of *Cryptosporidium parvum*, *Rotavirus*, *Escherichia (E) coli* and *Clostridium perfringens*. Stained stool samples were also examined for *Cryptosporidium* detection. Positive ones were excluded from the study. To prevent contact between lambs, each was housed separately in individual compartments.

2.2. Experimental formation of cryptosporidiosis

C. parvum oocysts needed for the formation of infection were obtained from rectal stool samples taken from naturally infected calves with diarrhea. Stool samples of the calves were examined using a rapid pen-side test (Bio-X Diagnostics S.A. Belgium) in terms of the presence of *Rotavirus, Coronavirus* and *E. coli* and negative ones were used. The oocysts required for infection formation were obtained as reported in a study conducted on goat kids (Koudela and Jiri, 1997).

For the formation of infection in lambs, 10 ml distilled water containing 10^6 *C. parvum* oocysts were given using an intraorally catheter (Nasogastric catheter 10 ch, 1210 mm, Bicakcilar Co. Ltd., Istanbul, TURKEY). The development of the infection was monitored through the collection of rectal stool samples daily and microscopic examination of the stained preparations. The stained preparations from stool samples was made according to Koudela and Jiri (1997). Lambs with an average of > 10 oocysts in stained stool preparations were considered to be infected, and treatment was initiated. When infection developed the following treatment protocols were applied to 4 groups, each including 8 lambs. Oocyst shedding started in the lambs on the 7th and 8th days, and it had reached to peak level between on day 10 and 13.

As a treatment procedure, doses of 100, 500 and 1000 mg/kg chitosan oligosaccharide were given to the lambs in 1st, 2nd, and 3rd groups at intervals of 12 h. No drug was given to the infected lambs in the 4th group and they were fed normally for positive control.

Considering dehydration degree and blood gas analysis findings, required fluid treatments were given to the lambs in which diarrhea caused dehydration developed (oral fluid treatment was applied to the lambs with mild or moderate dehydration and metabolic acidosis (pH = 7.20–7.35), intravenous 40 ml/kg/hour isotonic sodium bicarbonate was applied to the lambs with severe dehydration and metabolic acidosis (pH < 7.20), 5% dextrose was applied in case of hypoglycemia). During the study, lambs were fed with lamb food (Optimilk, Optima, Kirklareli, Turkey) at an amount of 10–12% of their body weight at a proper temperature in two meals. In addition, *ad-libitum* water was provided to lambs.

2.3. Properties of chitosan oligosaccharide

In the study, water-soluble (99%) chitosan oligosaccharide with a deacetylation degree > 90% and a molecular weight < 2000 daltons was used as the treatment material (GlycoBio Company, Dalian, China).

2.4. Clinical examinations

Routine clinical examinations of the lambs (body temperature, heart frequency, respiratory rate, capillary refill time, assessment of dehydration degree) were conducted before the treatment (day 0) and on 1st, 3rd, 5th and 7th days of the treatment. During the treatment, lambs were examined daily clinically and in terms of the stool analysis.

2.5. Collection of blood samples and analyses

5 ml of blood samples were collected from lambs before the treatment (day 0) and on 1st, 3rd, 5th and 7th days during treatment for blood gas analysis, hematological analyses (into K_3EDTA tubes) and biochemical analyses (into 5 ml anticoagulant free gel tubes).

2.6. Blood gas analyses

Blood hydrogen ion concentration (pH), partial carbon dioxide pressure (pCO_2), partial oxygen pressure (pO_2), bicarbonate (HCO_3^-), total carbon dioxide (TCO_2), base excess (BE), oxygen saturation (O_2sat), glucose and lactate levels were measured using blood gas analyzer (Epoc, Canada) immediately after blood sample collection without adding anticoagulant agents.

2.7. Hematological analyses

Using blood samples collected in K_3EDTA tubes, white blood cell (WBC), red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean cell hemoglobin concentration (MCHC), red cell distribution width (RDW) and platelet (PLT) values were determined by automatic cell counter (BC-2800VET, Mindray, China). Hematological analyses were conducted within 15 min after blood collection.

2.8. Biochemical analyses

The tubes without anticoagulant were kept at room temperature for clotting and centrifuged at 4000 rpm for 5 min to obtain serum. Serum samples were stored at -80 °C until the analysis. Urea, creatinine, total protein, albumin, aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT) and alkaline phosphatase (ALP) levels in serum samples were determined using autoanalyzer (BS 200, Mindray, China).

2.9. Examination of stool

Stool samples of lambs were collected from the rectum into sterile stool container before the treatment (day 0) and on the 1st, 3rd, 7th, 14th and 21st days of the treatment. Stool samples were evaluated in terms of physical [normal(solid), loose and formless, semi-fluid, watery] and oocysts. The presence of *C. parvum* oocysts in stools was determined using Kinyoun Acid-fast staining method (Korkmaz and Ok, 2011). Stained preparations were examined using immersion oil at 100X magnification in the light microscope. The concentration of *C. parvum* oocysts in the stool was averaged by counting the oocysts in 20 different microscope fields in each preparation, and semi-quantitative scoring was made as follows;

the absence of oocysts (0),
oocyst < 1 (1)
oocysts between 1–10 (2),
oocysts > 10 (3).

Lambs with a mean number of oocysts > 10 were included in the study (Koudela and Jiri, 1997).

Examination of stool samples were made by the same personnel.

2.10. Weight changes

Lambs were weighed using a digital scale before treatment (day 0) and on post-treatment 7th, 14th and 21st days and weight changes were recorded.

2.11. Statistical analysis

The data were expressed as mean and standard error of the mean (SEM). Kolmogorov-Smirnov test was used for normality. One-way ANOVA and Tukey multiple range tests were used to evaluate differences between each treatment group during the experiment and significance levels of variation. The statistical significance level was accepted to be $P < 0.05$. The SPSS software program (Version 15.0, SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

3. Results

After oral administration of oocysts, as of 3rd day, it was observed that there were changes in the characteristics of stool, that there was a decrease in the stool consistency and that oocyst excretion started with the presence of mucus. After oral administration of oocysts in lambs, as of 4th-5th days, diarrhea ranging from the pasty to liquid in consistency containing a high number of oocysts was observed and lambs that were suitable to the experimental procedure were taken to treatment protocols. Infection occurred in all lambs in 4 groups, and experimental procedure was applied to all lambs (n = 32). Stools with mucus were observed in 5, 4, 5 and 6 lambs in groups 1, 2, 3, and 4, respectively. When intense oocysts began to be excreted, stools were in yellow-greenish color and sometimes brown color and had a condensed mucus appearance. There was no blood observed in stools.

There was no death in groups 1–3 for 7 days; however, 1 lamb in group 4 died on the 3rd day and 1 lamb on the 7th day. Both lambs died in the 4th group had condensed mucus and liquid diarrhea and dehydration were observed in these lambs. Inappetence, abdominal distension, severe diarrhea and hypoglycemia were observed on the last day in the dead lamb. The lambs did not respond to fluid treatment applications and died.

3.1. Clinical findings

Lambs that developed cryptosporidiosis generally had a dynamic appearance, normal mucosa, and good sucking reflex. However, depression, reluctance to stand up, dehydration (max. 6% level), prolonged capillary refill time (3 s) and a decrease in sucking reflex were observed in some lambs. Dehydration occurred only in 5 of 32 lambs in a maximum level of 6%. Changes in the body temperature, heart and respiratory frequencies of lambs are presented in Table 1. The body temperature of the lambs was observed to range from 38.2 to 40.2 °C. There was a significant difference ($p < 0.05$) observed on day 0 between the groups in terms of body temperature and no significant difference was found for the other days. Moreover, the body temperature of lambs decreased in all groups compared to day 0 and this decrease was statistically significant except for the 4th group ($p < 0.05$). A significant difference was found on the 1st day between the groups in terms of respiratory frequency ($p < 0.05$).

3.2. Changes in oocyst excretion

Changes in the oocyst excretion of the lambs before the treatment (day 0) and on post-treatment 1st, 3rd, 5th, 7th, 14th and 21st days are given in Table 2. It was found that oocyst excretion in group 1 and 2 significantly decreased from the 3rd day compared to day 0 ($p < 0.05$). On the other hand, it was found that oocyst excretion in group 3 and 4 significantly decreased from the 5th day compared to day 0 ($p < 0.05$). As between groups, a significant decrease was observed in group 2 on the 3rd day and in group 3 on the 5th day compared to group 4 ($p < 0.05$).

3.3. Body weight changes

Body weight measurements of lambs were performed before the treatment (day 0) and on post-treatment 7th, 14th and 21st days. The changes in the body weight of the lambs by days are given in Table 3. It was found that lambs in all groups had weight loss on the 7th day and that there was a gradual increase in the weight on 14th and 21st days. There was no significant difference observed between groups in terms of weight changes of lambs. When the in-group differences between the days were examined, significant differences were found in the groups except for the third group ($p < 0.05$).

3.4. Blood gas analysis findings

The blood gas analysis results of the lambs before and during treatment by days are given in Table 4. In all groups, the mean blood pH was within the normal range (7.35–7.45) during the treatment. In-groups, there was no statistically significant change in blood pH between the days; however, there was a statistically significant difference between the groups on the 5th and 7th days ($p < 0.05$). Metabolic acidosis was observed in 3 cases in the group 1 on day 1 and 3; 1 case in the group 2 on day 0; 2 cases in the group 3 on day 0 and 1; 2 cases in the group 4 on day 5 and 7. While pCO_2 levels were similar between groups, increases and decreases were observed in-groups between the days. However, these increases and decreases were statistically significant only in 1st and 2nd groups ($p < 0.05$). Blood pO_2 and O_2sat levels did not show significant change between groups and in-groups between days. The lactate level of group 4 was significantly higher than the other groups on day 0 and the lactate level of group 2 was significantly higher than those of group 1 and group 4 on the second day. There were increases and decreases in lactate level in-group between days and a statistically significant difference was observed in 1st and 4th groups ($p < 0.05$). There were increases and decreases in HCO_3, BE and TCO_2 levels of lambs during the treatment. However, these changes were not significant to affect pH. The difference in HCO_3, BE and TCO_2 levels in-group between days was significant ($p < 0.05$) only in the 2nd group. There were significant differences determined between the groups in terms of HCO_3, BE and TCO_2 levels on 5th and 7th days ($p < 0.05$).

3.5. Hematological analysis findings

Changes in the hematological parameters of the lambs by days before and during the treatment are given in Table 5. Although there were increases and decreases in hematological parameters in-group between days, there was no statistically significant difference found. There was significant ($p < 0.05$) difference in WBC levels on the 1st day between the groups and there was no significant difference found on the other days. There was a similarity between the groups in terms of RBC, HGB, HCT, MCHC and PLT levels. In addition, between the groups, MCV levels were different at a statistically significant level on 0th, 1st and 3rd days and RDW levels on the 1st day ($p < 0.05$).

Table 1
Changes in clinical findings of the lambs with cryptosporidiosis (Mean ± SEM).

Parameters	Pre-treatment	Post-treatment (days)			
	0	1	3	5	7
Temperature (°C)					
Group 1	39.10 ± 0.15^{ab,A}	38.69 ± 0.24^{AB}	38.38 ± 0.24^{B}	38.51 ± 0.23^{AB}	38.94 ± 0.26^{AB}
Group 2	39.10 ± 0.21^{ab,A}	38.75 ± 0.19^{AB}	38.69 ± 0.13^{AB}	38.56 ± 0.16^{B}	38.55 ± 0.15^{B}
Group 3	39.19 ± 0.14^{a,A}	39.01 ± 0.11^{A}	38.39 ± 0.19^{B}	38.38 ± 0.12^{B}	38.60 ± 0.10^{B}
Group 4	38.66 ± 0.11^{b}	38.38 ± 0.29	38.26 ± 0.17	38.37 ± 0.18	38.26 ± 0.42
Heart rate (min)					
Group 1	130.00 ± 6.51	121.75 ± 4.20	129.25 ± 8.08	118.00 ± 5.61	116.25 ± 7.88
Group 2	120.00 ± 6.01	119.75 ± 10.27	117.38 ± 5.59	108.00 ± 7.56	102.00 ± 4.36
Group 3	129.88 ± 8.49	118.13 ± 7.10	120.00 ± 6.23	109.75 ± 8.81	110.50 ± 8.17
Group 4	130.75 ± 8.71	126.25 ± 7.75	123.14 ± 8.96	125.43 ± 11.30	117.14 ± 9.07
Respiration rate (min)					
Group 1	44.25 ± 4.96^{A}	37.00 ± 1.81^{b,AB}	34.75 ± 2.42^{AB}	30.25 ± 4.13^{B}	31.25 ± 2.67^{B}
Group 2	34.00 ± 3.91	32.63 ± 3.57^{ab}	31.63 ± 3.80	31.50 ± 2.56	30.00 ± 2.27
Group 3	40.50 ± 2.97	37.50 ± 6.02^{ab}	39.50 ± 3.66	33.00 ± 1.46	32.38 ± 2.66
Group 4	37.75 ± 2.66	41.75 ± 1.83^{a}	34.57 ± 2.57	36.29 ± 2.71	36.57 ± 4.20

Different letters in the same rows (A, B) and columns (a, b) are statistically significant ($P < 0.05$). Group 1: 100 mg/kg COS, Group 2: 500 mg/kg COS, Group 3: 1000 mg/kg COS at intervals of 12 h, Group 4: No drug was given to the infected lambs.

Table 2
Changes in the oocyst excretion of the lambs with cryptosporidiosis during treatment.

Groups	Pre-treatment		Post-treatment (days)				
	0	1	3	5	7	14	21
Group 1	3.00 ± 0.00^{A}	2.75 ± 0.16^{AB}	2.38 ± 0.18^{ab,B}	1.75 ± 0.16^{ab,C}	1.25 ± 0.25^{C}	0.29 ± 0.29^{D}	0.67 ± 0.21^{D}
Group 2	3.00 ± 0.00^{A}	2.88 ± 0.13^{A}	2.25 ± 0.16^{b,B}	1.88 ± 0.30^{ab,BC}	1.38 ± 0.32^{CD}	0.25 ± 0.16^{E}	0.86 ± 0.26^{DE}
Group 3	3.00 ± 0.00^{A}	2.75 ± 0.16^{A}	2.50 ± 0.19^{ab,A}	1.50 ± 0.19^{b,B}	1.50 ± 0.19^{B}	0.57 ± 0.30^{C}	0.71 ± 0.18^{C}
Group 4	3.00 ± 0.00^{A}	3.00 ± 0.00^{A}	2.86 ± 0.14^{a,A}	2.43 ± 0.30^{a,B}	1.86 ± 0.14^{C}	1.00 ± 0.00^{D}	1.00 ± 0.00^{D}

Different letters in the same rows (A, B, C, D, E) and columns (a, b) are statistically significant ($P < 0.05$). Group 1: 100 mg/kg COS, Group 2: 500 mg/kg COS, Group 3: 1000 mg/kg COS at intervals of 12 h, Group 4: No drug was given to the infected lambs.

Table 3
Body weight (kg) changes during the treatment of the lambs with cryptosporidiosis.

Groups	Pre-treatment Post-treatment (days) Post-treatment (days)			
	0	7	14	21
Group 1	4.23 ± 0.14^{B}	3.76 ± 0.13^{B}	3.98 ± 0.18^{B}	4.73 ± 0.23^{A}
Group 2	4.33 ± 0.22^{AB}	3.98 ± 0.23^{B}	4.33 ± 0.22^{AB}	4.68 ± 0.17^{A}
Group 3	4.22 ± 0.27	3.75 ± 0.26	4.19 ± 0.32	4.64 ± 0.34
Group 4	3.84 ± 0.32^{AB}	3.50 ± 0.33^{B}	4.22 ± 0.17^{AB}	4.52 ± 0.19^{A}

Different letters in the same rows (A, B) are statistically significant ($P < 0.05$). Group 1: 100 mg/kg COS, Group 2: 500 mg/kg COS, Group 3: 1000 mg/kg COS at intervals of 12 h, Group 4: No drug was given to the infected lambs.

3.6. Biochemical analysis findings

Changes in the biochemical parameters of the lambs by days before and during the treatment are given in Table 6. While AST levels of the lambs increased during the treatment compared to pre-treatment (day 0), ALP, TP and GGT levels decreased. However, only changes in AST and ALP activities of the 1st group and the change in total protein level of the 2nd group were statistically significant ($p < 0.05$). While there were increases and decreases in urea and creatinine levels of the groups by days, changes in the creatinine levels only in the 1st and 2nd groups were significant ($p < 0.05$). When the glucose levels of the groups were examined by days, glucose levels of the 1st, 2nd and 4th groups decreased on the 2nd day compared to day 0 and increased in the following days. The glucose level of the 3rd group decreased on the 1st and 3rd days compared to day 0 and increased gradually in the following days. Statistical significance was determined only in the 1st group ($p < 0.05$). Significant differences ($p < 0.05$) between the groups were determined only in TP and creatinine levels on the 7th day.

4. Discussion

A major problem about cryptosporidiosis is the lack of an effective tool for the prevention and treatment of this disease. More than 200 substances have been tested to treat cryptosporidiosis (Dinler and Ulutas, 2017). Some have shown promising effects; however, none of them have been reported to control clinical findings consistently or eliminate the infection completely. On the other hand, the use of certain drugs may reduce the oocyst excretion and thus probably the environmental pathogen level, the subsequent exposure, and infection of susceptible hosts (Shahiduzzaman and Daugschies, 2012). It has been reported that paromomycin, halofuginone lactate, lasalocid, and sulfaquinoxaline have partial or demonstrable effect against *Cryptosporidium* in infected neonatal ruminants (Wright and Coop, 2007; Aydogdu et al., 2018). However, knowledge about the treatment of cryptosporidiosis in sheep is limited. Halofuginone exhibits 2-fold toxicity of the recommended doses of lactate, and its use is contraindicated in severely dehydrated or inappetent animals (Wright and Coop, 2007). In addition, many drugs such as paromomycin, azithromycin, and sulfaquinoxaline that have been determined to reduce oocyst excretion in cryptosporidiosis have a risk to leave antibiotic residues. In recent years, serious concerns about antibiotic residues have emerged worldwide. In recent years, there have been studies carried out about the effects of chitosan and its derivatives on the underlying mechanism of antimicrobial activity of chitosan microparticles in the medical field and on the treatment of infectious diseases (Jeon et al., 2014). Kim et al. (2001) reported that oral chitosan oligosaccharide applications at doses of 500, 1000 and 2000 mg/kg/day did not cause any side effects

Table 4
Changes in blood gases during the treatment of the lambs with cryptosporidiosis.

Parameters	Pre-treatment	Post-treatment (days)			
	0	1	3	5	7
pH					
Group 1	7.42 ± 0.01	7.40 ± 0.02	7.37 ± 0.03	7.39 ± 0.02^{ab}	7.40 ± 0.02^{ab}
Group 2	7.43 ± 0.02	7.42 ± 0.01	7.42 ± 0.01	7.43 ± 0.01^{a}	7.43 ± 0.01^{a}
Group 3	7.40 ± 0.02	7.42 ± 0.02	7.43 ± 0.01	7.41 ± 0.01^{ab}	7.43 ± 0.01^{a}
Group 4	7.37 ± 0.03	7.39 ± 0.02	7.38 ± 0.02	7.37 ± 0.02^{b}	7.36 ± 0.03^{b}
pCO_2 (mm Hg)					
Group 1	46.35 ± 1.24^{AB}	44.10 ± 1.65^{B}	46.06 ± 1.77^{AB}	49.43 ± 1.71^{A}	50.09 ± 1.26^{A}
Group 2	43.85 ± 1.40^{B}	46.70 ± 2.02^{AB}	47.89 ± 1.71^{AB}	48.20 ± 1.11^{AB}	50.41 ± 1.11^{A}
Group 3	46.38 ± 2.34	44.15 ± 1.66	48.18 ± 1.88	49.48 ± 1.78	49.01 ± 2.00
Group 4	47.78 ± 1.27	46.11 ± 1.02	46.11 ± 1.02	47.00 ± 1.46	50.39 ± 2.12
pO_2 (mm Hg)					
Group 1	29.65 ± 3.08	33.83 ± 4.13	31.48 ± 2.38	31.51 ± 1.94	29.89 ± 1.37
Group 2	37.56 ± 5.39	28.49 ± 1.35	31.30 ± 2.25	28.43 ± 1.57	29.96 ± 1.74
Group 3	27.96 ± 1.67	34.49 ± 5.57	26.19 ± 1.26	30.00 ± 2.34	31.01 ± 2.49
Group 4	28.85 ± 1.99	31.70 ± 4.63	28.93 ± 2.36	34.83 ± 6.14	27.31 ± 3.20
Lactate (mmol/L)					
Group 1	2.10 ± 0.26^{b,A}	1.32 ± 0.19^{b,B}	1.42 ± 0.17^{B}	1.56 ± 0.23^{AB}	1.62 ± 0.20^{AB}
Group 2	1.74 ± 0.19^{b}	2.24 ± 0.44^{a}	1.60 ± 0.27	1.73 ± 0.25	1.67 ± 0.13
Group 3	1.60 ± 0.26^{b}	1.64 ± 0.25^{ab}	1.37 ± 0.23	1.93 ± 0.48	1.07 ± 0.18
Group 4	3.00 ± 0.36^{a,A}	1.37 ± 0.14^{b,B}	1.81 ± 0.53^{AB}	1.83 ± 0.51^{AB}	1.61 ± 0.45^{B}
HCO_3^-(mmol/L)					
Group 1	29.30 ± 0.75	27.19 ± 1.43	28.03 ± 2.04	30.08 ± 1.10^{a}	30.86 ± 1.29^{ab}
Group 2	29.30 ± 1.13^{B}	30.41 ± 1.50^{AB}	30.96 ± 1.19^{AB}	32.21 ± 0.58^{a,AB}	33.54 ± 0.53^{a,A}
Group 3	28.89 ± 1.71	28.90 ± 1.64	32.10 ± 1.51	31.46 ± 1.31^{a}	32.20 ± 1.25^{ab}
Group 4	28.51 ± 1.16	28.39 ± 1.23	28.84 ± 1.23	26.03 ± 1.95^{b}	28.77 ± 2.32^{b}
BE (mmol/L)					
Group 1	4.89 ± 0.73	2.31 ± 1.64	2.91 ± 2.53	5.15 ± 1.33^{ab}	6.03 ± 1.63^{ab}
Group 2	5.04 ± 1.38^{B}	5.96 ± 1.63^{AB}	6.45 ± 1.27^{AB}	7.96 ± 0.67^{a,AB}	9.23 ± 0.56^{a,A}
Group 3	4.13 ± 1.93	4.40 ± 1.92	7.79 ± 1.67	6.84 ± 1.42^{a}	7.84 ± 1.27^{ab}
Group 4	3.43 ± 1.48	3.51 ± 1.51	3.79 ± 1.51	2.20 ± 1.85^{b}	3.30 ± 2.79^{b}
TCO_2 (mm Hg)					
Group 1	30.70 ± 0.79	28.55 ± 1.47	29.46 ± 2.06	31.60 ± 1.12^{ab}	32.40 ± 1.27^{ab}
Group 2	30.66 ± 1.14^{B}	31.84 ± 1.55^{AB}	32.44 ± 1.24^{AB}	33.68 ± 0.59^{a,AB}	35.08 ± 0.54^{a,A}
Group 3	30.34 ± 1.75	30.29 ± 1.67	33.58 ± 1.56	32.99 ± 1.35^{a}	33.70 ± 1.31^{ab}
Group 4	29.96 ± 1.15	29.80 ± 1.25	30.34 ± 1.24	28.90 ± 1.56^{b}	30.33 ± 2.35^{b}
O_2sat (%)					
Group 1	54.86 ± 6.97	59.76 ± 6.30	56.11 ± 4.61	57.46 ± 4.37	54.96 ± 2.94
Group 2	64.66 ± 8.03	53.86 ± 3.67	58.96 ± 5.04	55.00 ± 4.17	57.03 ± 4.05
Group 3	51.13 ± 4.27	58.71 ± 7.46	48.86 ± 3.55	55.23 ± 5.05	58.13 ± 4.91
Group 4	51.58 ± 5.17	54.21 ± 6.62	52.03 ± 5.89	51.44 ± 10.51	48.99 ± 6.72

pH: concentration of hydrogen ions, pCO_2: partial pressure of carbon dioxide, pO_2: partial pressure of oxygen, HCO_3^-: bicarbonate, TCO_2; total amount of carbon dioxide, BE: base excess, O_2sat: oxygen saturation. Different letters in the same rows (A, B) and columns (a, b) are statistically significant ($P < 0.05$). Group 1: 100 mg/kg COS, Group 2: 500 mg/kg COS, Group 3: 1000 mg/kg COS at intervals of 12 h, Group 4: No drug was given to the infected lambs.

in rats. In a study conducted, protective activity of chitosan oligosaccharide has been determined in mice in which colitis was formed experimentally (Yousef et al., 2012). Chung et al. (2012), found that oral administering of chitosan oligosaccharide with a low molecular weight reduced the allergic inflammation in mice in which experimental asthma model was formed. Abdel-Latif et al. (2016) reported that it had "anticoccidial" effects on Eimeria papillata-infected mice. In the same study, it has been reported that both excreted oocysts and developmental stage parasites were reduced and chitosan had anti-inflammatory activity (Abdel-Latif et al., 2016). In a study, oral chitosan oligosaccharide administering in the treatment of diarrheal calves has been reported to be very successful (Alam et al., 2012). In addition, chitosan has been used to create excipients in a microparticle system and it has been reported that it might play a role as a physical barrier against *Cryptosporidium parvum* (Blanco-García et al., 2016). In the studies conducted, it has been reported to inhibit the *in vitro* development of *C. parvum* (Luzardo Álvarez et al., 2012; Adjou et al., 2014; Mammeri et al., 2018). In addition, Mammeri et al. (2018) reported that chitosan NAG and chitosan mix demonstrated in vivo anticryptosporidial properties in CD-1 mice, a highly sensitive animal model against *C. parvum* infection. Therefore, it has been stated that both of these compounds have a promising potential in therapeutic and preventive applications against *C. parvum* infection. In this study, oocyst excretion decreased significantly ($p < 0.05$) in the 1st and 2nd groups by the 3rd day compared to pre-treatment and in the 3rd and 4th groups by the 5th day. As between groups, a significant decrease was observed in group 2 on the 3rd day and in group 3 on the 5th day compared to group 4 ($p < 0.05$). Although stool characteristics became normal on the 14th and 21 st days, it was observed that oocyst excretion still continued in the lambs. While all the lambs in the positive control group had oocyst excretion at the level of +1 on the 14th and 21st days, this level was found to be lower in treatment groups compared to the positive control group. However, there was no statistical difference found. These results showed that chitosan oligosaccharide significantly reduced oocyst excretion compared to the positive control group; however, it was not efficacious to eliminate cryptosporidiosis completely. According to the findings of this study, the use of chitosan oligosaccharide to be used in the treatment of experimental cryptosporidiosis at doses of 100 and/or 500 mg/kg was found to provide an earlier reduction in oocyst excretion compared to 1000 mg/kg dose. The use of higher doses had no negative effect; however, it increases the costs and does not show more positive affects compared to lower doses. Considering this, a dose of 100 mg/kg was thought to be the most appropriate dose. The results indicated that low dose of chitosan oligosaccharide was better to reduce oocyst excretion and improve clinical findings in lambs with cryptosporidiosis. Studies

Table 5
Changes in hematological parameters of the lambs with cryptosporidiosis during treatment.

Parameters	Pre-treatment	Post-treatment (days)			
	0	1	3	5	7
WBC (10^9/L)					
Group 1	13.00 ± 2.98	13.48 ± 1.47^{a}	11.36 ± 2.63	11.61 ± 1.94	14.40 ± 2.39
Group 2	11.61 ± 1.59	9.94 ± 1.25^{ab}	10.68 ± 1.38	12.20 ± 1.38	11.51 ± 1.04
Group 3	10.60 ± 2.62	8.51 ± 1.32^{b}	9.53 ± 1.43	9.98 ± 1.68	9.86 ± 1.64
Group 4	11.99 ± 2.22	10.86 ± 0.87^{ab}	8.78 ± 1.66	9.33 ± 1.58	11.27 ± 1.91
RBC (10^{12}/L)					
Group 1	10.47 ± 0.96	11.66 ± 0.51	11.88 ± 0.82	11.51 ± 0.72	10.82 ± 0.32
Group 2	11.21 ± 0.73	11.24 ± 0.55	11.51 ± 0.44	11.02 ± 0.60	10.11 ± 0.62
Group 3	10.91 ± 0.77	10.75 ± 0.59	11.46 ± 1.05	11.65 ± 0.73	10.49 ± 0.51
Group 4	10.52 ± 0.65	10.70 ± 0.52	10.49 ± 0.53	10.21 ± 0.87	9.43 ± 0.77
HGB (g/dL)					
Group 1	13.35 ± 1.14	15.13 ± 0.81	15.29 ± 1.58	14.74 ± 1.26	13.29 ± 0.59
Group 2	14.31 ± 1.25	14.25 ± 1.02	14.36 ± 0.70	13.56 ± 0.97	12.20 ± 0.94
Group 3	13.96 ± 0.91	13.74 ± 0.71	14.71 ± 1.51	14.86 ± 1.14	12.74 ± 0.80
Group 4	14.64 ± 1.09	15.11 ± 0.94	14.30 ± 1.17	14.33 ± 1.92	12.67 ± 1.41
HCT (%)					
Group 1	39.70 ± 3.19	45.13 ± 2.38	46.60 ± 3.72	43.74 ± 3.65	40.66 ± 1.71
Group 2	43.93 ± 3.68	43.74 ± 2.76	42.78 ± 2.44	41.63 ± 2.81	37.86 ± 2.67
Group 3	42.25 ± 2.15	41.09 ± 1.82	42.85 ± 3.86	44.03 ± 3.01	39.69 ± 2.07
Group 4	45.60 ± 3.30	45.79 ± 2.80	44.96 ± 3.76	43.41 ± 5.22	39.07 ± 4.30
MCV (fL)					
Group 1	38.53 ± 1.18^{b}	38.76 ± 1.18^{b}	39.19 ± 1.01^{ab}	38.94 ± 1.81	37.66 ± 1.22
Group 2	38.89 ± 0.91^{b}	38.78 ± 0.78^{b}	38.40 ± 0.77^{b}	38.01 ± 0.87	37.21 ± 0.72
Group 3	39.24 ± 1.04^{b}	38.98 ± 1.13^{b}	38.95 ± 1.18^{b}	38.96 ± 1.29	39.33 ± 1.61
Group 4	43.11 ± 1.41^{a}	42.76 ± 1.35^{a}	42.59 ± 1.71^{a}	41.89 ± 1.68	40.97 ± 1.63
MCHC (g/dL)					
Group 1	33.19 ± 0.45	33.40 ± 0.26	33.54 ± 0.26	33.38 ± 0.20	32.88 ± 0.23
Group 2	32.48 ± 0.23	33.51 ± 0.40	32.41 ± 0.36	32.48 ± 0.33	32.10 ± 0.50
Group 3	32.78 ± 0.73	32.81 ± 0.50	32.83 ± 0.84	32.64 ± 0.72	32.06 ± 0.60
Group 4	32.16 ± 0.66	33.00 ± 0.78	31.81 ± 0.56	32.69 ± 0.91	32.39 ± 0.93
RDW (%)					
Group 1	19.01 ± 0.84	19.11 ± 0.69^{ab}	18.30 ± 0.65	19.06 ± 0.71	18.84 ± 0.73
Group 2	18.13 ± 0.30	17.90 ± 0.39^{b}	18.01 ± 0.39	17.89 ± 0.32	18.01 ± 0.36
Group 3	19.83 ± 0.68	19.91 ± 0.81^{a}	20.01 ± 0.76	19.89 ± 0.74	19.74 ± 0.65
Group 4	19.05 ± 0.62	18.85 ± 0.56^{ab}	19.13 ± 0.88	19.09 ± 0.88	18.96 ± 0.95
PLT (10^9/L)					
Group 1	794.13 ± 124.52	845.75 ± 137.02	1016.38 ± 123.98	924.50 ± 116.74	823.38 ± 85.73
Group 2	924.25 ± 142.48	923.38 ± 84.07	814.50 ± 134.50	927.75 ± 96.75	831.25 ± 109.13
Group 3	752.38 ± 116.05	754.63 ± 87.65	844.38 ± 101.26	845.25 ± 80.82	757.00 ± 87.23
Group 4	848.88 ± 159.93	887.13 ± 165.17	1038.71 ± 155.43	917.43 ± 85.96	769.86 ± 95.26

WBC; white blood cell, RBC; red blood cell, HGB: hemoglobin, HCT: hematocrit, MCV: mean corpuscular volume, MCHC: mean cell hemoglobin concentration, RDW: red cell distribution width; PLT: platelet. Different letters in the same rows (A, B) and columns (a, b) are statistically significant ($P < 0.05$). Group 1: 100 mg/kg COS, Group 2: 500 mg/kg COS, Group 3: 1000 mg/kg COS at intervals of 12 h, Group 4: No drug was given to the infected lambs.

(Mammeri et al., 2018) on the exact mechanism of action of chitosan continue. It has been reported that it is possible for chitosan to cause effects on the production of trophozoites and oocysts via various mechanisms (direct effects on parasitic viability and/or infectivity and/or parasitic growth in intestines and/or oocyst formation) (Mammeri et al., 2018). However, further studies are required to determine the exact mechanism of action. The limitations of this study are the absence of a negative control group without infection and treatment and the absence of groups treated with COS without cryptosporidiosis infection.

Generally, if there is no mix infection, the clinical findings in cryptosporidiosis are not severe and are transient and mild levels. In mix infections, clinical findings are more severe and mortality rates are higher. Morbidity rates are high and mortality is low in cryptosporidiosis infections alone (Constable et al., 2017). In this study, it was observed that the changes in the clinical findings were not severe in all groups; however, there were some individual differences. The characteristics of diarrhea improved day by day in the treatment groups and in the positive control group. In the treatment groups (G1–3), stools became normal or pasty in consistency on the 7th day, while there was still diarrhea in liquid level in 2 cases in the positive control group on the 7th day. These results showed that chitosan oligosaccharide provided faster recovery in clinical findings and stool characteristics in lambs with cryptosporidiosis compared to the positive control group.

The most important clinical finding in cryptosporidiosis is diarrhea in varying levels. Endogenous forms of *Cryptosporidium* lead to loss of mature enterocytes, shortening, and fusion of villus. They disrupt the microvilli boundary which causes the elongation of crypts caused by increased cell division and edema. This causes the loss of membrane-bound digestive enzymes, reduces intestinal absorption capacity and reduces intake of liquids, electrolytes, and nutrients from the intestinal lumen (Foster and Smith, 2009; Constable et al., 2017). In this study, it was found that although some significant in-group and/or intragroup changes were observed in blood gases, hematological and biochemical parameters in both treatment groups and in the positive control group, these changes remained limited and progressed generally within the reference limits. Metabolic acidosis was observed in 3 cases in the group 1 on day 1 and 3; 1 case in the group 2 on day 0; 2 cases in the group 3 on day 0 and 1; 2 cases in the group 4 on day 5 and 7. However, it was determined that metabolic acidosis was at moderate and severe levels in only 1 st and 4th groups in 1 case in each. In other cases, the severity of metabolic acidosis remained mild. These results show that experimentally developed cryptosporidiosis alone in lambs leads to changes in blood gases and hematological and biochemical parameters; however, these changes were not at a level to disrupt the general condition.

In conclusion, it was found that experimental cryptosporidiosis can

Table 6
Changes in biochemical parameters of the lambs with cryptosporidiosis during treatment.

Parameters	Pre-treatment	Post-treatment (days)			
	0	1	3	5	7
AST (U/L)					
Group 1	78.13 ± 7.49^{B}	81.00 ± 9.08^{B}	91.50 ± 9.48^{AB}	106.25 ± 8.02^{AB}	115.88 ± 5.62^{A}
Group 2	87.38 ± 10.94	99.38 ± 13.68	104.88 ± 10.93	113.50 ± 8.65	128.00 ± 13.44
Group 3	83.71 ± 16.89	99.14 ± 21.27	112.71 ± 20.84	126.86 ± 20.20	146.43 ± 18.27
Group 4	97.50 ± 8.52	112.38 ± 8.10	128.00 ± 11.04	140.57 ± 24.43	150.57 ± 37.86
ALP (U/L)					
Group 1	547.75 ± 58.09^{A}	514.63 ± 51.94^{AB}	451.63 ± 42.81^{AB}	382.00 ± 25.37^{AB}	368.88 ± 22.43^{B}
Group 2	568.13 ± 95.59	528.13 ± 72.22	493.88 ± 68.48	409.75 ± 51.70	369.63 ± 52.58
Group 3	642.43 ± 100.73	561.29 ± 74.72	498.00 ± 65.54	427.43 ± 37.17	395.71 ± 38.33
Group 4	559.25 ± 61.17	486.25 ± 61.24	472.29 ± 48.53	470.14 ± 76.11	446.00 ± 54.27
GGT (U/L)					
Group 1	221.38 ± 80.44	147.38 ± 2.79	164.88 ± 48.19	143.00 ± 33.90	134.00 ± 27.39
Group 2	285.88 ± 114.31	251.50 ± 89.33	214.38 ± 68.06	184.75 ± 52.33	161.50 ± 42.08
Group 3	270.86 ± 54.50	256.57 ± 74.34	164.43 ± 27.09	146.71 ± 21.56	134.86 ± 18.15
Group 4	304.63 ± 66.25	212.00 ± 47.67	235.29 ± 49.67	209.86 ± 38.00	240.00 ± 40.26
TP (g/dL)					
Group 1	5.39 ± 0.62	4.58 ± 0.59	4.89 ± 0.55	4.88 ± 0.54	4.53 ± 0.55^{ab}
Group 2	6.06 ± 0.46^{A}	4.80 ± 0.32^{AB}	4.88 ± 0.43^{AB}	4.94 ± 0.52^{AB}	4.14 ± 0.36^{b,A}
Group 3	5.74 ± 0.52	5.67 ± 0.48	5.23 ± 0.51	5.10 ± 0.47	4.91 ± 0.46^{ab}
Group 4	6.06 ± 0.55	5.43 ± 0.48	5.83 ± 0.35	5.79 ± 0.57	5.87 ± 0.43^{a}
Albumin (g/dL)					
Group 1	2.71 ± 0.08	2.75 ± 0.07	2.73 ± 0.06	2.75 ± 0.06	2.75 ± 0.06
Group 2	2.74 ± 0.03	2.71 ± 0.04	2.66 ± 0.05	2.70 ± 0.05	2.74 ± 0.04
Group 3	2.79 ± 0.10	2.79 ± 0.10	2.73 ± 0.07	2.77 ± 0.06	2.77 ± 0.10
Group 4	2.59 ± 0.14	2.65 ± 0.07	2.70 ± 0.04	2.63 ± 0.06	2.64 ± 0.11
Creatinine (mg/dL)					
Group 1	0.69 ± 0.08^{B}	0.89 ± 0.10^{AB}	0.98 ± 0.12^{AB}	1.02 ± 0.10^{A}	0.86 ± 0.07^{ab,AB}
Group 2	0.66 ± 0.05^{B}	0.82 ± 0.06^{AB}	0.97 ± 0.08^{A}	0.91 ± 0.12^{A}	1.03 ± 0.07^{a,A}
Group 3	1.26 ± 0.32	0.85 ± 0.07	0.91 ± 0.07	0.92 ± 0.04	0.92 ± 0.10^{ab}
Group 4	0.90 ± 0.15	0.80 ± 0.09	0.79 ± 0.10	0.72 ± 0.11	0.69 ± 0.11^{b}
Urea (mg/dL)					
Group 1	22.88 ± 4.67	22.56 ± 4.47	32.24 ± 3.50	33.60 ± 3.44	31.18 ± 2.10
Group 2	20.54 ± 2.19	23.18 ± 2.17	23.89 ± 2.18	26.33 ± 2.34	27.06 ± 1.86
Group 3	25.31 ± 4.82	27.34 ± 5.59	25.46 ± 3.65	33.01 ± 3.81	34.28 ± 4.90
Group 4	32.27 ± 7.65	35.99 ± 7.47	30.39 ± 5.95	30.95 ± 4.60	25.37 ± 2.58
Glucose (mg/dL)					
Group 1	108.50 ± 8.79^{AB}	83.00 ± 6.35^{B}	99.25 ± 6.27^{AB}	111.63 ± 11.23^{AB}	135.50 ± 21.19^{A}
Group 2	109.38 ± 9.12	93.00 ± 5.33	96.88 ± 12.87	97.38 ± 11.78	113.50 ± 15.50
Group 3	97.50 ± 12.98	92.25 ± 8.98	76.00 ± 8.59	86.25 ± 12.69	92.50 ± 15.39
Group 4	105.25 ± 15.21	72.13 ± 9.84	87.14 ± 10.60	91.57 ± 14.75	96.50 ± 22.00

AST: aspartate aminotransferase, ALP: alkaline phosphatase, GGT: gamma-glutamyltransferase, TP: total protein. Different letters in the same rows (A, B) and columns (a, b) are statistically significant ($P < 0.05$). Group 1: 100 mg/kg COS, Group 2: 500 mg/kg COS, Group 3: 1000 mg/kg COS at intervals of 12 h, Group 4: No drug was given to the infected lambs.

be formed successfully through the oral inoculation of the solution containing 10^6 oocysts in lambs aged < 5 days, that oral administering of chitosan oligosaccharide in lambs at doses of 100, 500 and 1000 mg/kg had no side effect, that chitosan oligosaccharide provided faster recovery in clinical findings and stool characteristics in lambs in which cryptosporidiosis was formed experimentally compared to positive control group and that administering of chitosan oligosaccharide at doses of 100, 500, 1000 mg/kg for 7 days significantly decreased oocyst excretion; however, it was not efficient enough to completely eliminate cryptosporidiosis. In this study, the appropriate chitosan oligosaccharide dose to treat cryptosporidiosis in lambs was observed to be 100–500 mg/kg, whereas new studies on this subject are required. We think that this study will provide a basic knowledge for further studies on the use of chitosan and its derivatives in the treatment of cryptosporidiosis in veterinary medicine.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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