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THE EFFECTS OF ORALLY GIVEN HIGH RATE CARBOHYDRATE ON SOME PATHOGENS THAT PLAY AN IMPORTANT ROLE IN ETIOLOGY OF THE DIARRHEA IN CALVES

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Abstract. This study was carried out on 30 adult calves which completed their rumen development aged from three to six months old in the public held. Twenty calves received totally 1500 ml of propylene glycol per day 3-5 times/day, assigned as study group, while 10 calves that were found to be clinically healthy and didn't receive any additional treatments served as control group. Clinical, stool, hematological and blood biochemical examinations were performed in all the animals. At the end of the study; it had been found that propylene glycol caused mild diarrhea (15%) and some slightly respiratory system problems (10%), decreased the number of calves which showed fecal-pathogenic agents in their feces, and didn't cause significant problems in the liver. Consequently; it was determined that a glucose precursor propylene glycol could be used safely in adult calves, and it might help to reduce fecal contamination to neonates by decreasing the number of fecal pathogens, besides increased productivity.

Key words: Calves; Propylene glycol; Pathogenic agents; Faeces.

INTRODUCTION

Calf diarrhea is still the most frequent and significant economic loss in cattle breeding, despite the big improvements in herd management, housing conditions, care, nutrition and biopharmaceuticals (Izzo, M.M. et al. 2011). These studies report that while calf deaths in the neonatal period are higher than in the adult turnover, adult deaths are also at a significant level.

Despite the large number of studies carried out at early ages, the health of older adults calves is less researched. Studies of morbidity have shown that the diarrhea and respiratory diseases are the most important disease groups seen in the older calves (Perez, E. et al. 1990; Olsson, S.O. et al. 1993; Svensson, C. et al. 2003). As a matter of fact, the incidence of diarrhea is decreasing with age (Frank, N.A., Kaneene, J.B. 1993; Bendali, F. et al. 1999), while the risk of developing diarrhea in the first months of life is low.

Propylene glycol (PG) is one of the most widely used substances for energy supply in cattle, decrease in the amount of ketone bodies, increase in yield and elimination of losses during disease (Gordon, J.L. et al., 2017; Raboisson, D. et al. 2014; Gohary, K. et al. 2016; Bjerre-Harpoth, V. et al. 2016). The use of high doses of PG may lead to diarrhea as well as toxic effects (Fiume, M.M. et al. 2012). Sabbioni, A. et al. 1999) reported that long-term administration of PG (50 ml/animal/ day) in the high dose was caused to slightly toxic effects in the liver as well as diarrhea formation.

The aim of this study was to investigate the effects of higher dose of PG on the presence of pathogenic agents (virus, bacteria, protozoa) in the feces as well as diarrhea formation in calves in their older ages.

MATERIALS AND METHODS

Animal Material:

The study was carried out on 30 calves who developed rumen, three to six months old in public held. Twenty calves were recieved 1500 ml of propylene glycol per day 3-5 times/day in total, assigned as the study group (ÇG), while 10 of the calves were found to be clinically healthy and without additional treatment served as control (KG).

The present study was carried out within the framework of ethical rules of the Ethical Committee of Animal Experiments of Afyon Kocatepe University with the reference number of AKUHADYEK 197-17, 17. CAREER. 69 and the Afyon Kocatepe University Scientific Research Projects Coordination Unit (BAPK).

Clinical Examinations:

Body temperature, respiration and heart rates, ruminal contractions in 5 minutes along with diarrhea, dehydration and appetite control were determined using the methods described by Blood and Radostits, (1989).

Hematological Examination:

Hematological parameters such as total leukocyte (WBC), erythrocyte (RBC), mean corpuscular volu-

me (MCV), hematocrit (HCT), mean cell hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), hemoglobin were measured by Hemocellcounter (Mindray Hemocell Veterinary Model).

Blood Biochemical Tests:

Some blood parameters such as aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), glucose (GLU), albumin (ALB) and total protein (TP were measured on Roche Cobas C111 model autoanalyzer using commercial kits.

Viral and Parasitological Experiments:

The commercial Rapid Test Kit (Quatro Vet Uni-Strip Kit, C-1540, Coris BioConcept, Belgium) was used to verify rotavirus, coronavirus, cryptosporidium, giardia and *E. coli* in fecal samples. The presence of *Eimeria spp.* in the stool was made using the native examination method of fresh faeces (Blood and Radostits, 1989).

Statistical Analyzes:

One-way ANOVA and Duncan's test were used to test differences between groups. Also, Repeated Measures ANOVA was used for repeated measures in comparing the measurements at different times for the same individuals. The level of significance was determined as 0.05 in the analyses made in the study and SPSS 18.0 for Windows package program was used in the analysis of the obtained data.

Results

Clinical, hematological and biochemical examination findings with the presence of pathogens in the faeces of animals in the study and control group are shown as below.

Existence of Patients in the Stool and Clinical Examination Findings:

Findings related to the presence of pathogens, dehydration, diarrhea and loss of appetite before and after the application of propylene glycol (PG) in the study group (SG) animals are shown in Graphic 1, in relation to the parameters mentioned in the Control Groups (CG) the data are shown in Graphic 2.

The animals in the SD received a gradual increase in the level of diarrhea following the administration of PG and the highest number (15% of the animals) on day 5, the last day of the study. Similarly, dehydration and loss of appetite reached 5% of all animal populations (n= 20) on day 5, although observed in different animals. From the point of view of CG animals, no change was found in terms of the parameters mentioned. Interestingly, there were detected in 2 animals (10% of the entire polution) of the animals (n = 20), coronavirus, cryptosporidium and *E. coli*, and 3 (15%) Eimeria chart 1) in SG. On the days following PG administration, a reduction in fecal and efficacious animal numbers was observed, with the lowest numerical value being determined on the fifth day of the last day of administration. Compared to the pre-administration effluent, only 5% of the total animals (n = 1) of pathogenic microorganisms were found to have reduced half-life (50%) on the 5th day after the administration of PG, and the route and corona virus, cryptosporidium and *E. coli* pathogens 1 animal. Similarly, the number of animals found in Eimeria in their stools decreased by 30% on the 5th day.,10% of total animals), cryptosporidium (20% of total animals), *E. coli* (20% of total animals) and Eimeria (10% of total animals) (20% of total animals), 3 animals (corresponding to 33% of the total animals) were found in the mix (Graph-2). There was no numerical change in the duration of the study from the point of the CG animals in terms of smearing of excreta.

The clinical parameters measured in CG and SG animals are shown in Table 1.

When Table 1 is examined; SG animals were observed to be significantly higher ($p < 0.05$) in the statistically significant ($p < 0.05$) level of pre-administration and body temperature of the CG animals, within the normal limits, following days of PG administration. However, no statistically significant difference was found between recalcitrant days of CG animals and the average of the animals before PG administration ($p > 0.05$). There was no statistically significant difference between consecutive days averages of SC animals after PG administration ($p > 0.05$). A similar statistic was also found in terms of the given heart and respiratory frequencies. Although the respiratory and cardiac frequencies of all CG and SG animals were within normal limits, there were no statistically significant changes between the QoL intervals and the pre-PG averages of PG animals ($p > 0.05$) ($p > 0.05$), but the PG administration was significantly higher ($p < 0.05$) in the statistically significant difference between the respiratory and cardiac frequency averages before and after the administration of the CG animals, within the normal range on the following days. In addition, 2 animals (10%) were found to have a problem of respiratory air, australic lung sounds hardened, mild respiratory system problem. Although it led to a slight increase

in the number of rumen contractions of the PG for 5 minutes, it was still statistically insufficient to make a statistically significant difference (p> 0.05) in terms of the rumen contractions at 5 min during the study period in CG and SG animals.

Hematological Examination Findings:

Avarage rates of hematological findings observed in this study are shown at Table 2.

When Table 2 is examined; it was determined that the highest WBC, RBC, HB and HCT averages (7.90 ± 2.10, 8.60 ± 3.00, 9.90 ± 2.40, 30.60 ± 4.20) were obtained on the 5th day after PG administration. When the comparisons between the groups were examined, it was understood that CG did not show any difference between the mean values determined for the parameters mentioned in all the time periods of animals and the mean of the pre-PG data before the application (p> 0.05). The mean values obtained after the PG administration in the SG animals were significantly higher than the mean values of the PG animals before and after the PG administration in terms of statistical significance (p> 0.05), although the mean values of the SG animals in terms of the stated parameters were not statistically significant (p> 0.05). In terms of MCV, MCH and MCHC levels measured in this study, there was no statistically significant change in terms of time intervals between groups (p> 0.05).

Metabolic Profile Findings:

The averages of the data obtained from blood biochemical specimens in this study are shown in Table 3.

According to this Table the highest average values of AST and GGT enzyme levels were formed on the 5th day after PG administration (86.50 ± 15.30, 296.70 ± 68.30, respectively) and gradually increased from day 1 to day 5 after PG administration, (p <0.05) was found to be statistically significant. Similarly, it was found that the mean values of the SG animals were significantly higher (p <0.05) in terms of statistical significance than the mean of CG before PG and PG, but there was a statistically significant difference between the mean values of PG and PG pre-PG averages (p> 0.05). Similar, but more pronounced, elevation measurements have also been observed in terms of GLU concentrations. There was no statistically significant difference (p> 0.05) in terms of the GLU concentration averages of SG animals before CG and PG administration (p> 0.05) and the mean values obtained at all time intervals of CG were higher than the average of SG animals before PG administration. Similar elevations were also observed in EC animals after the PG administration and the difference between the average of GLU concentration levels in the days following PG administration was statistically significant (p <0.05), the highest level was found on the 5th day after PG administration (98.47 ± 7.26). In terms of TP and ALB concentration levels, there was no significant difference between both groups (p> 0.05).

Discussion

In our literature reviews, we unfortunately did not find a lot of works that directly addressed the effects of PG on diarrhea in adult icebergs and possible pathogens that are spread by feces in this diarrhea. However, PG, a carbohydrate percursor, is known to cause diarrhea by altering osmotic pressure in the digestive tract (Hammer et al., 1989; Hendrickson, 2017; Trabue et al., 2007). In our present study, it was determined that diarrhea developed in 3 (15%) of the ED animals at the 3rd day following PG administration, whereas no diarrhea was detected before the first mailling of the CG animals and before the PG administration of the SG animals. which can lead to the formation of the nature.

As it is well known, even if pathogenic agents do not form the disease table on adult calves, they continue to be found in facultative form in the digestive tract flora in healthy calves (Janke, B.H. 1989; Fagan, J.G. et al. 1995; Uhde, F.L. et al. 2008). In our work a total of 1 animal rota and corona virus were found in CG, 2 animals Eimeria, 2 *E. coli* and 2 cryptosporidium were detected. In SG animals, after 2 weeks of PG administration, a total of 2 animal rota virus, 1 animal corona virus, 2 animals Eimerai, 2 cryptosporidium and 3 *E. coli* were found to be in agreement with the researchers.

Recently, PG has been the most carefully studied glucogenic supplements, and some researchers (Robinson, E. and Sprayberry, K. 2009) mention that antibacterial and antifungal effects may be present. T.O. Thorgeirsdottir et al. (2003) reported that PG increased antiviral efficacy in combination with antiviral drugs at different concentrations. T.M. Nalawade et al. (2015) claimed that PG had bacteriocidal effects on many bacteria, especially E. coli. M. Khaw et al. (1995) reported an increase in the activity of antiprotozoal drugs used in combination with PG. As a matter of fact, the decrease in the number of animals showing these factors in the feces in the days after the PG application in our current study, espe-

cially on the 5th day, especially on the last day, supports the finds of the researchers even if the number is small.

As a result of PG administration at high doses; (Nielsen, N.I. et al. 2004; McClanahan, S. et al. 1998; Ivany, J.M. and Anderson, D.E. 2001), as well as respiratory system-related disorders such as depression, ataxia, excessive salivation, malodorous respiration, and malodorous fecal symptoms. As a matter of fact, although in our study, we could not mention a numerical calf population enough to support this data following the PG application, it was found that all the factors were detected at the same time, 1 of the animals in the SG had a bad smell of respiratory air and 2 animals had a slight garlic-like odor its appearance, supports the above-mentioned views.

The present study supports the findings of researchers (Munday, R. and Manns, E. 1994) who reported that the lowest levels of HGB and RBC, as well as the mean levels of HGB and RBC, were detected on the 5th day of the SG and that oxidative stress-related hemolytic anemia could be formed in animals given PG-like substances.

PG, a glucose precursor, is often used to relieve energy needs. Frequent use of PG does not produce toxic effects (Fiume, M.M. et al. 2012; Ivany, J.M. and Anderson, D.E. 2001; DeFrain, J.M. et al. 2014), it can also cause diarrhea. Along with the diarrhea table; hypovolemia (dehydration and loss of metabolites), metabolic acidosis, hyperkalemia, renal insufficiency (Cho, Y. and Yoon, K. 2014; Steven et al. 2007). The higher levels of GLU levels measured in our study compared to the KG average after PG administration support the findings of these investigators. A. Sabbioni et al. (1999) reported that blood triglycerides and NEFA decreased, while long-term use improved mild toxicity in the liver, while providing PG (50 ml/animal/day) carcass increase in adult icebergs. In our study, PG was applied for 5 days and the AST measured in the icebergs supports the GGT enzyme levels reported by these researchers, which are higher than the average of CG, while staying within normal limits. Emery et al.,1964), on the other hand, argue that even with high doses such as 2000 g/cow/day, PG does not produce any side effects or toxicity data. In our study, the measurement of blood results from biochemical investigations is closer to normal than that reported by this investigator.

Consequently; PG, a commonly used carbohydrate precursor, causes little diarrhea as a result of a 5-day trial, causing some increases in some of the liver's enzymes, within normal limits, but these elevations are not enough to claim a toxicity. In addition, it has been concluded that PG causes a numerical decrease in fecal and efficacious animal numbers and that it would be useful to use it on adult icebergs.

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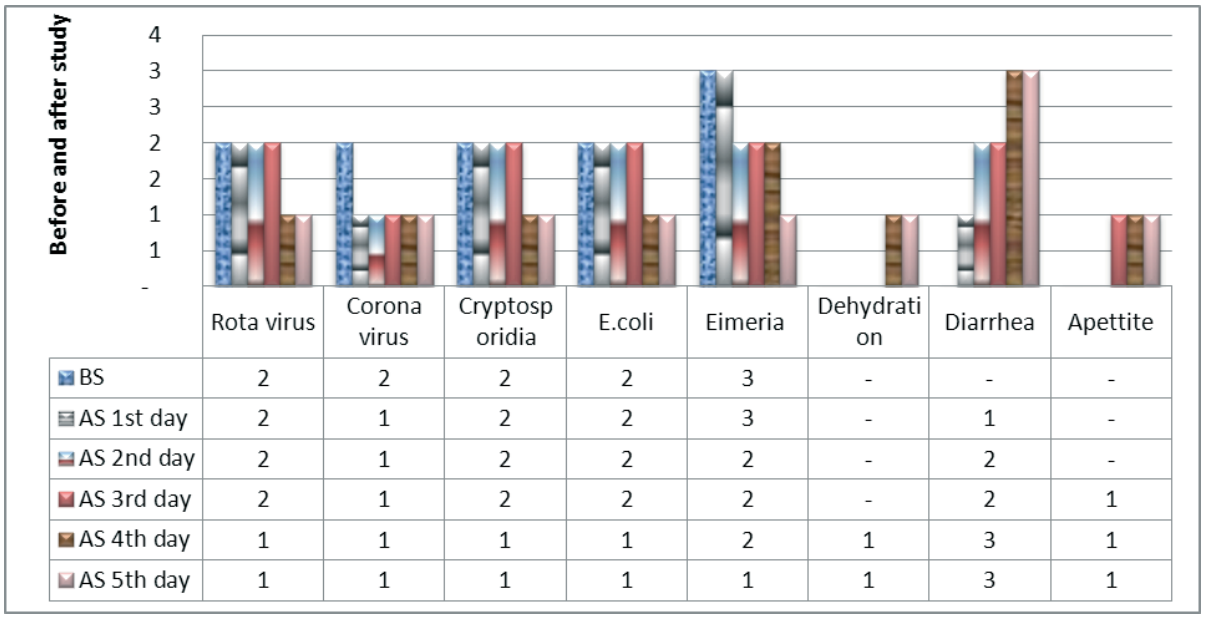
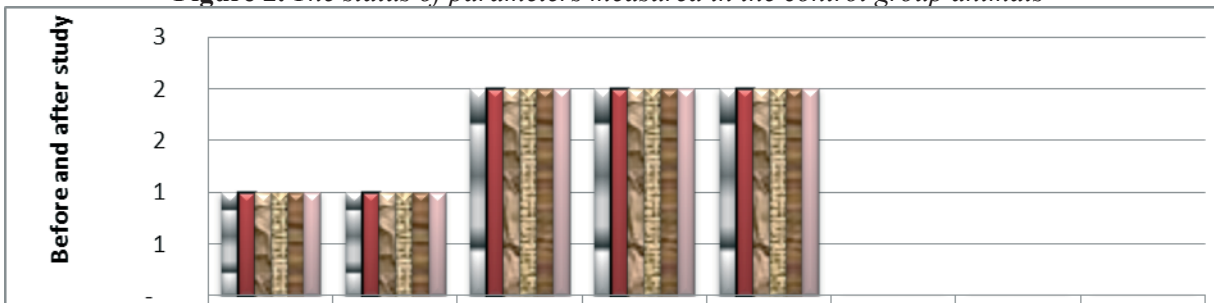
Figure 1. *The status of parameters measured in the study group animals*



	Rota virus	Corona virus	Cryptosp oridia	E.coli	Eimeria	Dehydrati on	Diarrhea	Apettite
BS	2	2	2	2	3	-	-	-
AS 1st day	2	1	2	2	3	-	1	-
AS 2nd day	2	1	2	2	2	-	2	-
AS 3rd day	2	1	2	2	2	-	2	1
AS 4th day	1	1	1	1	2	1	3	1
AS 5th day	1	1	1	1	1	1	3	1

*BS: Before study, AS: After study

Figure 2. *The status of parameters measured in the control group animals*



	Rota virus	Corona virus	Cryptosp oridia	E.coli	Eimeria	Dehydrati on	Diarrhea	Apettite
BS	1	1	2	2	2	-	-	-
AS 1st day	1	1	2	2	2	-	-	-
AS 2nd day	1	1	2	2	2	-	-	-
AS 3rd day	1	1	2	2	2	-	-	-
AS 4th day	1	1	2	2	2	-	-	-
AS 5th day	1	1	2	2	2	-	-	-

*BS: Before study, AS: After study

Table 1. *Average body temperature, heart and respiratory, and rumen contraction frequency statistics*

Groups/ Parametrs	T (°C)	P (min)	R (min)	Ruminal contraction (in 5 mins)
CG (n=10)				
	X±SD	X±SD	X±SD	X±SD
BS	38.20± 0.20	82.00± 4.00^{b}	34.00±3.00^{b}	10.00±4.00
AS 1st day	38.10± 0.20	80.00± 5.00^{b}	34.20± 3.00^{b}	11.00±3.00
AS 2nd day	38.20± 0.10	81.20± 4.00^{b}	33.20± 2.20^{b}	11.00±2.00
AS 3rd day	38.40± 0.20	83.00± 5.20^{b}	34.00± 4.00^{b}	11.00±3.00

AS 4th day	38.20± 0.20	82.00± 5.00^{b}	33.40± 3.40^{b}	10.00±4.00
AS 5th day	38.30± 0.20	83.20± 4.00^{b}	34.00± 4.00^{b}	11.00±3.00
SG (n=20)				
BS	38.30± 0.30	82.20± 3.00^{b}	34.20±2.00^{b}	12.00±3.00
AS 1st day	38.30± 0.30	84.50± 4.00^{a}	36.40± 4.00^{a}	12.00±2.00
AS 2nd day	38.40± 0.30	86.40± 4.00^{a}	37.30± 3.00^{a}	11.00±0.00
AS 3rd day	38.30± 0.40	85.40± 5.00^{a}	36.40± 3.00^{a}	12.00±2.00
AS 4th day	38.40± 0.40	86.20± 4.00^{a}	37.00± 4.00^{a}	12.00±3.00
AS 5th day	38.50± 0.50	86.40± 5.00^{a}	37.20± 4.00^{a}	12.00±2.00

^{a,b} The difference between the averages of the control groups carrying different letters in the same column is important in terms of statistics ($p<0.05$).
SG: Study group, CG: Control group

Table 2. *Hematological examination data averages of CG and SG animals*

Time	Grup	WBC (m/mm3)	RBC (m/mm3)	HB (g/dl)	HCT %	MCV(fl)	MCH (pg)	MCHC (g/dl)
		X±SD	X±SD	X±SD	X±SD	X±SD	X±SD	X±SD
BS	**CG**	6.28±160^{b}	7.30±1.60^{b}	8.30±2.20^{b}	26.00±3.40^{b}	35.40±3.30	11.40±1.20	32.10±2.40
	SG	6.34±1.30^{b}	7.30±1.80^{b}	8.40±2.30^{b}	26.10±3.30^{b}	35.50±3.30	11.50±1.30	32.20±2.30
AS 1st day	**CG**	6.40±1.30^{b}	7.30±1.40^{b}	8.30±2.20^{b}	26.40±3.60^{b}	36.20±3.40	11.40±1.30	31.50±2.40
	SG	6.90±2.20^{b}	7.50±1.70^{b}	8.80±2.40^{ab}	27.30±3.30^{b}	36.50±3.40	11.70±1.40	32.20±2.60
2nd day	**CG**	6.38±1.30^{b}	7.30 ±1.40^{b}	8.20±2.30^{b}	26.50±3.40^{b}	36.40±3.60	11.30±1.50	31.50±2.40
	SG	7.10±2.20^{ab}	7.80±1.80^{ab}	9.00±2.40^{a}	29.10±4.00^{a}	37.20±3.50	11.50±1.50	31.00±2.50
3rd day	**CG**	6.38±1.30^{b}	7.30±1.38^{b}	8.30±2.40^{b}	26.30±3.10^{b}	36.10±3.50	11.40±1.30	31.50±2.60
	SG	7.70±2.20^{a}	8.10±2.36^{ab}	9.40±2.60^{a}	30.20±4.40^{a}	37.30±3.60	11.60±1.30	31.30±2.40
4th day	**CG**	6.42±1.10^{b}	7.30±1.28^{b}	8.10±2.10^{b}	26.30±3.20^{b}	36.10±3.30	11.10±1.40	31.90±2.30
	SG	7.80±2.30^{a}	8.30±2.86^{a}	9.80±2.60^{a}	30.60±4.00^{a}	37.00±3.20	11.80±1.20	32.10±2.40
5th day	**CG**	6.40±1.00^{b}	7.40±1.60^{b}	8.30±2.30^{b}	26.60±3.20^{b}	36.10±3.40	11.30±1.50	31.40±2.60
	SG	7.90±2.10^{a}	8.60±3.00^{a}	9.90±2.40^{a}	30.60±4.20^{a}	35.60±3.40	11.50±1.40	32.30±2.40

^{a,b} The difference between the averages of the control groups carrying different letters in the same column is important in terms of statistics ($p<0.05$).
SG: Study group, CG: Control group, BS: Before study, AS: After study

Table 3. *Average of metabolic profile data of CG and SG animals*

Time	Group	AST (IU/L)	GGT (IU/L)	TP (g/dl)	ALB (g/dl)	GLU (mg/dl)
		X±SD	X±SD	X±SD	X±SD	X±SD
BS	**CG**	72.40±12.30^{b}	242.20±36.30^{b}	5.30±1.10	3.02±0.24	62.50±4.30^{e}
	SG	72.30±12.00^{b}	243.40±40.60^{b}	5.40±1.20	3.06±0.13	62.40±4.18^{e}
AS 1st day	**CG**	72.60±14.00^{b}	239.60±38.20^{b}	5.30±1.40	3.08±0.14	63.22±4.08^{e}
	SG	72.40±13.00^{b}	242.60±42.40^{b}	5.40±1.30	3.05±0.16	69.12±5.34^{d}
2nd day	**CG**	74.20±14.40^{b}	244.20±50.20^{b}	5.48±1.36	3.04±0.12	63.28±4.24^{e}
	SG	78.90±16.30^{ab}	246.30±40.00^{b}	5.60±1.20	3.03±0.16	78.90±6.27^{c}
3rd day	**CG**	73.40±13.00^{b}	243.50±36.00^{b}	5.55±1.56	3.05±0.16	64.23±4.10^{e}
	SG	82.20±14.40^{a}	247.40±48.20^{b}	5.48±1.28	3.08±0.14	84.34±6.42^{b}
4th day	**CG**	73.30±14.60^{b}	245.40±40.80^{b}	5.24±1.32	3.04±0.16	65.43±5.28^{e}
	SG	85.40±15.40^{a}	286.20±60.20^{a}	5.38±1.16	3.08±0.14	95.48±8.25^{ab}
5th day	**CG**	74.30±14.00^{b}	244.80±44.60^{b}	5.45±1.28	3.06±0.12	64.43±5.28^{e}
	SG	86.50±15.30^{a}	296.70±68.30^{a}	5.70±1.34	3.08±0.13	98.47±7.26^{a}

^{a,b,c,d,e} The difference between the averages of the control groups carrying different letters in the same column is important in terms of statistics ($p<0.05$).
SG: Study group, CG: Control group, BS: Before study, AS: After study