Oregano essential oil administration diminishes the imbalances of certain serum biochemical variables in diarrheic calves

PANAGIOTIS DIMITRIOS KATSOULOS[1]*, ANNA DEDOUSI[2], MARIA ANASTASIA KARATZIA[3], ELENI GEORGIOU KATSOGIANNOU[4], LABRINI VASILEIOU ATHANASIOU[4]

[1] Clinic of Farm Animals, School of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece
[2] Veterinary Research Institute, Hellenic Agricultural Organization – Dimitra, Thessaloniki, Greece
[3] Research Institute of Animal Science, Hellenic Agricultural Organization – Dimitra, Paralimni Giannitsa, Greece
[4] Department of Medicine, Faculty of Veterinary Medicine, University of Thessaly, Karditsa, Greece

SUMMARY

Neonatal diarrhea syndrome is the most common disease occurring in calves aged less than 15 days and is often accompanied by dehydration, pre-renal azotemia and electrolyte imbalances. The objective of this study was to investigate under field conditions whether the daily administration of oregano essential oil could ameliorate the imbalances of certain biochemical parameters observed in calves with neonatal diarrhea syndrome: Thirty-seven diarrheic calves that did not require intravenous administration of fluids until recovery were divided into two groups; Eco group (n=23) calves were orally drenched with Greek oregano (*Origanum vulgare ssp. Hirtum*) essential oil at the dose of 12.5 mg/kg body weight once per day for the first 10 days of their life whereas those of Conts group (n=14) were left untreated and served as controls. All animals were monitored daily for the incidence of diarrhea and their fecal score was recorded. Blood samples were collected by jugular venipuncture at the age of 48h (D2) and 24h after the recovery from diarrhea (D1PD): Mean serum concentrations of albumin, blood urea nitrogen (BUN), creatinine, potassium (K), and inorganic phosphorus (P) were significantly increased ($P<0.05$) during diarrhea episodes whereas the concentration of total calcium (Ca) and the activity of aspartate aminotransferase (AST) was significantly decreased ($P<0.05$). On D1PD, the average serum concentrations of albumin, BUN, creatinine, K, and P were significantly lower and the concentration of Ca was significantly higher in ECO group compared to the controls: These results indicate that oregano essential oil administration could prevent the impairment of renal function of neonatal diarrheic calves and ameliorate the serum K, P and Ca imbalances.

KEY WORDS

Oregano essential oil; calves; neonatal diarrhea; serum biochemical; imbalances.

INTRODUCTION

Diarrhea syndrome is the most common clinical entity encountered in neonatal calves aged less than 15 days and a significant cause of impaired productivity and financial loss to cattle industry worldwide[1,2]. The morbidity rate may exceed 25% to 50% in poorly managed farms and the mortality rate ranges between 2% and 10%[3]. The primary infectious agents associated with the disease, enterotoxic *Escherichia coli* K99/F5, Rotavirus A, Bovine coronavirus, and *Cryptosporidium* spp.[4,5,6], directly affect the intestinal epithelial cells, resulting in diarrhea[7]. This, in turn, decreases the absorption of essential nutrients from milk, leading to weight loss[8], and increases the loss of water and electrolytes resulting in dehydration, pre-renal azotemia[9] and electrolyte imbalances[3].

Evidence in the literature suggests that diarrheic calves often have increased serum concentrations of creatinine and blood urea nitrogen (BUN) which are indicative of reduced glomerular filtration rate of pre-renal etiology [9,10,11,12] and decreased serum glucose concentration[9,11,12,13]. Hyponatremia is a very common electrolyte imbalance observed[10,14] but hypernatremia might also be detected if calves are over-treated with oral rehydrating solutions[15], especially with those that have excessive sodium concentration[16]. It is well documented that calves with diarrhea have a clear negative potassium balance due to intestinal losses and decreased milk intake[17]; many of them are hypokalemic[13] nevertheless, some are hyperkalemic despite the total body potassium depletion[18,19,20]. It has been proven that the latter is closely related to the degree of dehydration and results from the reduced renal excretion of potassium due to hypovolemia[20]. Hyperphosphatemia which is detected in some calves with diarrhea is also attributed to the same mechanism [18.20]. It is referred that electrolyte imbalances may still be present even 10 days after completion of therapy in calves successfully treated for diarrhea[16].

It is expected that treating diarrheic calves with agents, other than antibiotics, which contribute to the reduction of the duration and severity of diarrhea, along with the use of oral rehydrating solutions, could ameliorate these serum biochemical alterations. Oregano essential oil could be used as such an agent since it has been proven to be effective in diminishing the severity of naturally acquired diarrhea under field conditions, when administered to calves from the first day of their life on-

Corresponding Author:
Katsoulos Panagiotis Dimitrios (katsoulo@vet.auth.gr).

wards[21]. It was observed that the duration of diarrhea was shorter and the number of animals that needed advanced treatment was significantly lower in the group of calves that were orally drenched with oregano essential oil than the controls. However, the impact of this treatment on the biochemical alterations of diarrheic calves has not been evaluated yet.

The objective of the present study was to investigate under field conditions whether the daily administration of oregano essential oil could ameliorate the imbalances of certain biochemical parameters observed in calves with neonatal diarrhea syndrome.

MATERIALS AND METHODS

Thirty-seven diarrheic calves that did not receive any advanced treatment (IV fluids +/- antibacterials) until recovery were used for the purpose of the present study. These calves came from an initial population of 91 newborn calves used in a previous experiment[21]. According to the protocol, they had been assigned into two groups as follows: The animals of Eco group (n=46) were orally drenched with Greek oregano (Origanum vulgare ssp. hirtum) essential oil (ECODIAR® liquid 5%; Ecopharm Hellas S.A., Kilkis, Greece) at the dose of 12.5 mg/kg body weight once per day for the first 10 days of their life whereas those of Conts group (n=45) were left untreated and served as controls. The calves of Eco group were orally drenched immediately after the first colostrum feeding and the following days after the morning feeding with the respective amount of oregano essential oil that was diluted with normal saline up to the volume of 60 ml with the aid of a feeding syringe of equal volume. These calves were weighed on days 3, 6, and 10 of their life to ensure the appropriate daily dosage of oregano essential oil.

Fecal scoring was performed daily until the age of 15 days after the morning feeding by the same person using a three-point scale (1=normal, 2=intermediate and 3=watery). Calves with fecal scores ≥2 were considered diarrheic. Based on these records, the number of days with diarrhea (fecal score ≥2) and the average fecal score during diarrhea were determined.

During diarrhea, the animals were clinically evaluated daily until recovery by the same person who was blinded at group allocation. The dehydration status was evaluated as described by Constable et al. (1998). The calves finally included in the present study had normal rectal temperature, dehydration rate <8%, did not show any depression and retained their appetite throughout this period. They did not receive any other treatment except from oral electrolytes (Diaproof K®; Virbac, Hellas) offered as an extra meal per day between milk feedings until the normalization of feces (fecal score = 1). In calves of Eco group that were still diarrheic after day 10, administration of oregano essential oil continued until recovery.

Diarrhea was recorded in 32 out of 46 calves of Eco group (69.6%) and 41 out of 45 calves of Conts group (91.1%)[21]. Nine calves of Eco group and 27 of Conts group needed advanced treatment (IV fluids +/- antibacterials)[21]. These calves were excluded from the present study and the data of 23 calves of Eco group and 14 of Conts group were finally used.

Blood samples were collected from all calves 48h after birth (D2) and 24h after recovery (D1PD). The samples were obtained before the morning feeding via jugular venipuncture (21 G) into evacuated glass tubes and transferred refrigerated in the laboratory. After clotting, serum was separated by low-speed centrifugation (1600 g for 15 min) and transferred into plastic vials and forwarded for analysis. Serum concentrations of albumin (Alb), urea nitrogen (BUN), creatinine, glucose, total calcium

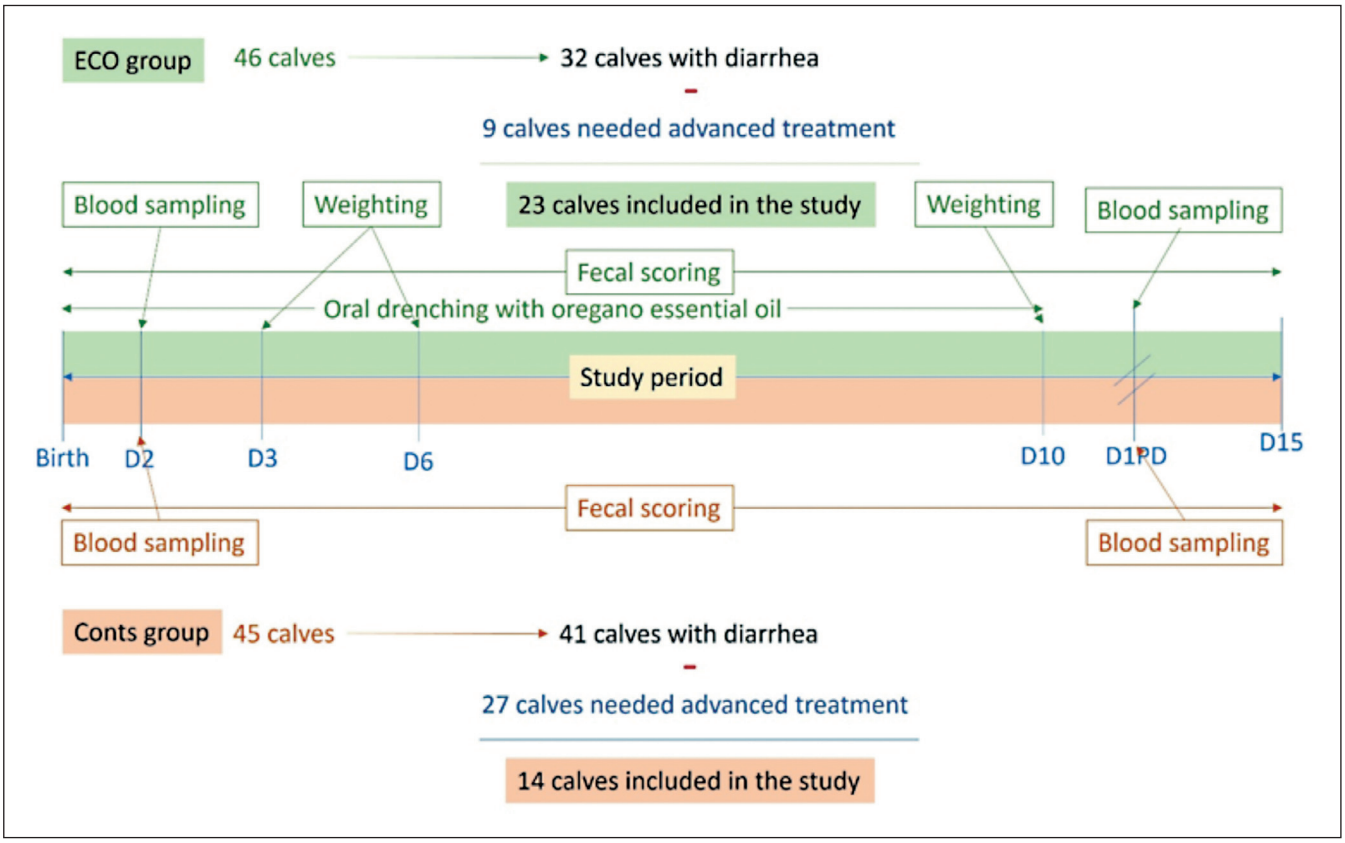

Figure 1 - Study design. D2, D3, D6, D10, D15: days 2, 3, 6, 10 and 15, respectively; D1PD: 24h after recovery from diarrhea.

(Ca), inorganic phosphorus (P) and the serum activities of aspartate aminotransferase (AST) and creatine kinase (CK), as well as potassium and sodium concentration were determined in an automatic chemistry analyzer (ADVIA 1800 Chemistry System, Siemens Healthineers) using the corresponding commercial diagnostic kits.

The study design and the selection of animals are presented in detail in Figure 1.

All procedures were done according to the ethical standards in the Helsinki Declaration of 1975, as revised in 2000, as well as the national law and the guidelines of our Institutional Animal Care and Use Committee. Ethical review and approval were waived for this study since sampling was performed only twice for diagnostic evaluation and no research on animals, as defined in the EU Ethics for Researchers document[22], was conducted. Informed consent was provided by animal owners regarding using results and animal data for research purposes.

Data were analyzed using the statistical program JASP 14.1. Normality of data distribution was assessed with the Shapiro-Wilk test and homogeneity of variances was evaluated with Levene test. The data for the number of days with diarrhea and the average fecal score during diarrhea were not normally distributed and Mann-Whitney test was used to determine the significance of the differences of these parameters among groups. Repeated measures ANOVA was run to evaluate the effect of sampling day (day) and of oregano essential oil administration (group) on the biochemical parameters evaluated. Post-hoc comparisons were done with Bonferroni test. A value of $P \leq 0.05$ was considered significant in all comparisons.

RESULTS

The median number of days with diarrhea was not significantly different between groups (3.00 and 4.00 days for ECO and Conts group, respectively; $P>0.05$). However, the median fecal score was significantly lower in Eco group compared to Conts (2.33 and 2.63 for ECO and Conts group, respectively; $P<0.05$).

Serum creatinine concentration was significantly affected by treatment and was significantly lower in ECO group compared to the controls ($P<0.05$; Table 1). It was also significantly affected by time and was significantly higher on D1PD in comparison with D2 ($P<0.05$; Table 1). A significant increase of serum creatinine concentration between D2 and D1PD was observed within Conts group ($P<0.05$; Figure 2a) whereas this increase was insignificant in ECO group ($P>0.05$; Figure 2a). The average values recorded on D1PD were significantly lower in ECO group compared to the controls ($P<0.05$; Figure 2a). On D1PD, 4 out of 14 (28.57%) animals of Conts group and 1 out of 23 (4.35%) calves of ECO group had serum creatinine values higher than the upper reference range (133 umol/l) but the difference was not significant ($P>0.05$). However, the percentage of animals with creatinine concentration higher than 100 mmol/l was significantly higher ($P<0.05$) in Conts group (57.14%) than ECO group (17.39%).

BUN concentration was significantly affected by time and was higher on D1PD in comparison with D2 ($P<0.05$; Table 1). As it is shown in Figure 2b, this increase was significant only within Conts group ($P<0.05$) and not within ECO group ($P>0.05$). In addition, on D1PD BUN was significantly higher in Conts group than ECO ($P<0.05$; Figure 2b). On this day, BUN values higher than the upper reference limit (10.9 mmol/l) was observed on the same calves (4 in Conts group and 1 in ECO group) that had high serum creatinine concentration in both groups.

Serum K and P concentrations were significantly affected by time and were significantly higher on D1PD compared to D2 ($P>0.05$; Table 1); this increase was significant only within Conts group ($P<0.05$; Figure 3). On D2PD, the average values recorded in ECO group were significantly lower ($P<0.05$) compared to the controls for both parameters (Figure 3). Treatment had a significant effect on serum K concentrations, with the lower values to be observed in ECO group ($P<0.05$; Table 1) whereas serum P concentrations remained unaffected ($P>0.05$; Table 1).

Serum Alb and Ca concentrations were significantly affected by both treatment and time ($P<0.05$; Table 1). However, they followed the opposite trend; serum Alb was significantly lower in ECO group compared to the controls and in D2 than D1PD whereas serum Ca was significantly higher than Conts in both comparisons (Table 1). As it is shown in Figure 4, serum Alb was increased significantly ($P<0.05$) and serum Ca was decreased significantly ($P<0.05$) within Conts group, and their concentrations were significantly different among groups on D1PD ($P<0.05$).

Table 1 - Mean±SE of biochemical parameters in calves drenched with oregano essential oil (Eco) and those left untreated (Conts) 48h after birth (D2) and 24h after recovery from diarrhea (D1PD).

	ECO	Conts	D2	D1PD	Reference range
Alb (g/l)	32.78±0.79^{a}	35.30±0.79^{b}	32.56±0.63^{A}	35.50±0.63^{b}	25 – 37
Glucose (mmol/l)	5.19±0.21^{a}	5.16±0.21^{a}	5.27±0.24^{A}	4.98±0.24^{A}	3.9 – 8.5
BUN (mmol/l)	4.47±0.52^{a}	5.27±0.52^{a}	3.71±0.50^{A}	6.03±0.55^{B}	2.7 – 10.9
Creatinine (umol/l)	76.58±3.59^{a}	96.42±3.59^{b}	76.81±3.19^{A}	96.19±3.19^{B}	56 – 133
Ca (mmol/l)	2.44±0.04^{a}	2.32±0.04^{b}	2.47±0.04^{A}	2.29±0.04^{B}	2.0 – 3.0
P (mmol/l)	2.05±0.06^{a}	2.21±0.06^{a}	2.03±0.05^{A}	2.22±0.05^{B}	1.3 – 2.6
K (meq/l)	5.30±0.14^{a}	5.82±0.14^{b}	5.27±0.13^{A}	5.85±0.13^{B}	3.5 – 5.5
Na (meq/l)	134.88±0.67^{a}	134.72±0.67^{a}	135.30±0.58^{A}	134.29±0.58^{A}	132 – 152
AST (U/L)	45.31±2.76^{a}	52.34±2.76^{a}	52.72±2.61^{A}	43.93±2.61^{B}	19 – 55
CK (U/l)	94.48±27.52^{a}	136.84±27.52^{a}	144.90±27.11^{A}	86.42±27.11^{A}	0 – 400

^{A,B,a,b} Different superscripts between groups (a,b) or between days (A,B) denote significant difference at $P \leq 0.05$; Alb: albumin; BUN: blood urea nitrogen; Ca: total calcium; P: inorganic phosphorus; K: potassium; Na: sodium; AST: aspartate aminotransferase; CK: creatine kinase.

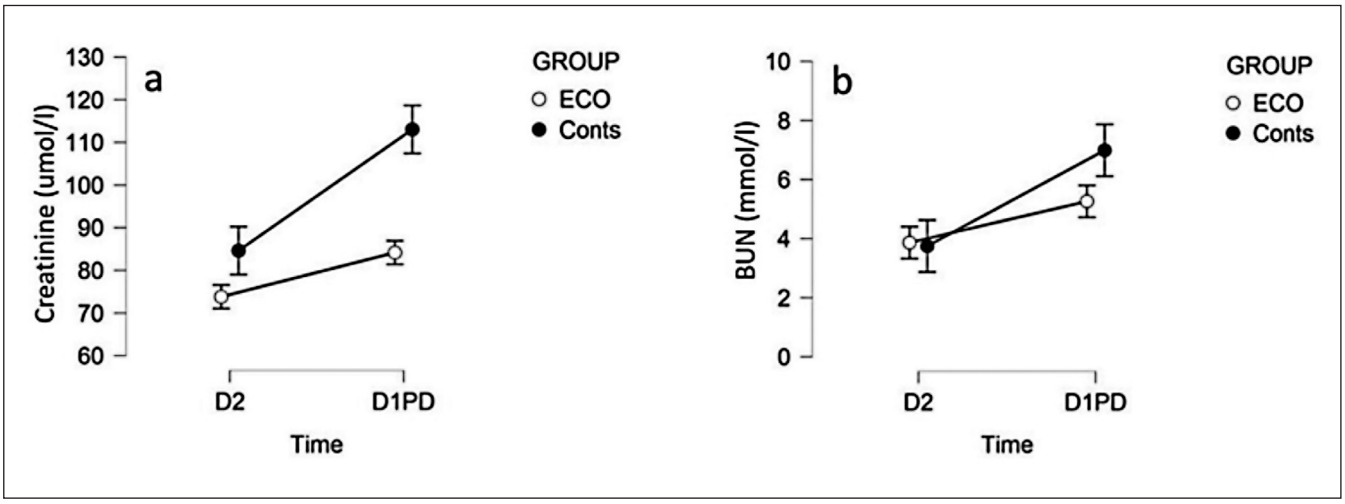

Figure 2 - Mean±SE of serum concentrations of creatinine (a) and blood urea nitrogen (BUN;b) in the group of animals that received oregano essential oil (Eco) and those left untreated (Conts) 48h after birth (D2) and 24h after recovery from diarrhea (D1PD).

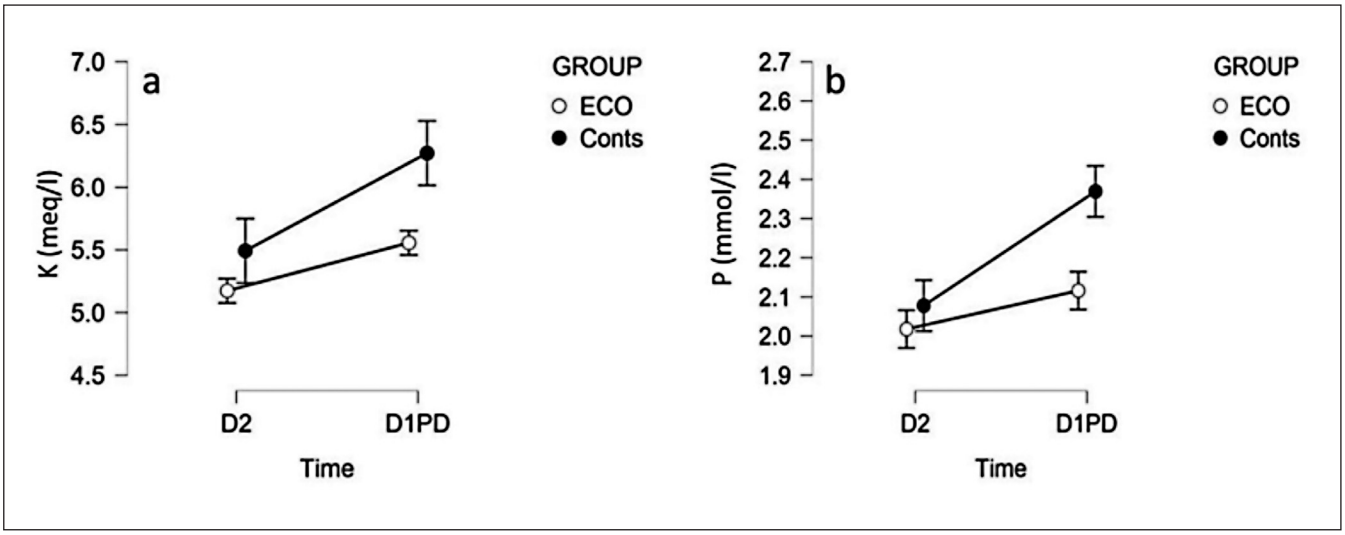

Figure 3 - Mean±SE of serum concentrations of potassium (K; a) and inorganic phosphorus (P; b) in the group of animals that received oregano essential oil (Eco) and those left untreated (Conts) 48h after birth (D2) and 24h after recovery from diarrhea (D1PD).

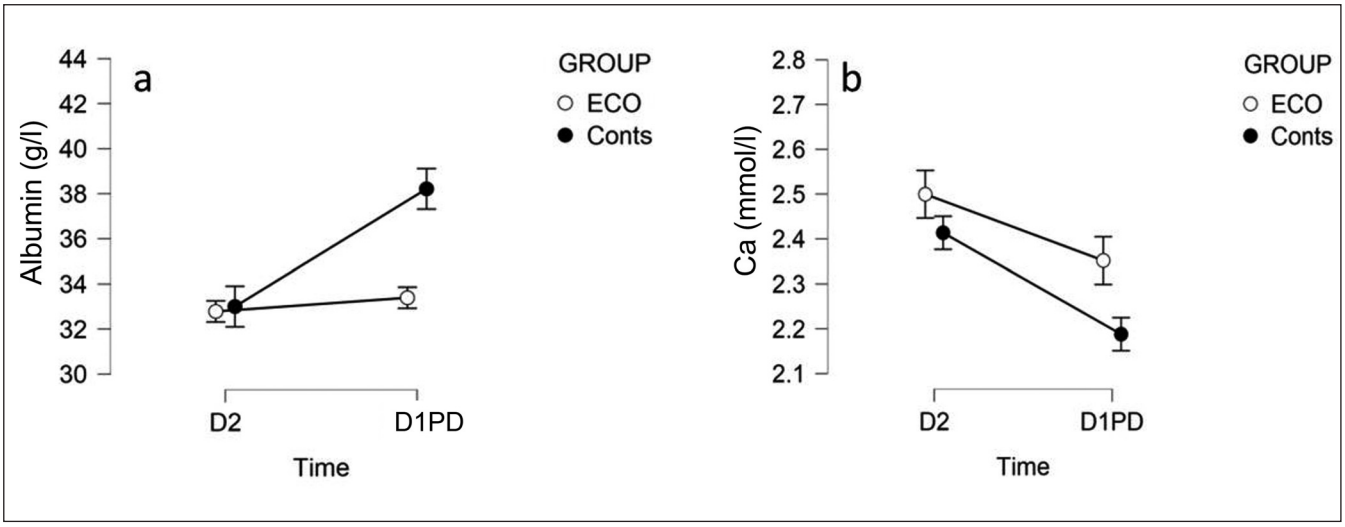

Figure 4 - Mean±SE of serum concentrations of albumin (a) and total calcium (Ca; b) in the group of animals that received oregano essential oil (Eco) and those left untreated (Conts) 48h after birth (D2) and 24h after recovery from diarrhea (D1PD).

Serum glucose and Na concentrations were not affected either by treatment or by time (P>0.05; Table 1). In addition, their values remained unaffected (P>0.054) between D2 and D1PD within each group.

Concerning the activities of the enzymes evaluated, none of them was significantly affected by the administration of oregano essential oil (P>0.05; Table 1). Time significantly affected AST activity and the values recorded were significantly reduced between D2 and D1PD. However, the alterations observed within both groups were not significant (P<0.05). Serum activity of CK was unaffected by time and remained practically stable between D2 and D1PD (P<0.05; Table 1).

DISCUSSION

The objective of this study was to investigate under field conditions, whether the administrations of oregano essential oil could ameliorate the biochemical imbalances observed during neonatal diarrhea syndrome in calves. It was selected to include in this study only the animals that did not receive any intravenous fluids throughout the diarrhea course. Such a selection might have led to an underestimation of the actual effects of oregano essential oil on the parameters evaluated since the percentage of animals that received advanced treatment in ECO group was significantly lower than the controls[21]. However, it was judged as necessary in order for the results not to be biased by the administration of treatment.

A very common biochemical abnormality observed in diarrheic neonatal calves is pre-renal azotemia which is characterized by increased BUN and creatinine concentrations[9,10,11,12]. This abnormality was detected in both groups at the present study, but the incidence was much lower in ECO group compared to the controls. Azotemia is attributed to hypovolemia and the consequent reduced renal blood flow and glomerular filtration rate due to dehydration[9]. The significant increase of serum creatinine and BUN concentrations only within Conts group and, especially, the significantly higher serum creatinine concentration in Conts compared to ECO group on D1PD proves that calves receiving oregano oil were able to maintain a more efficient glomerular filtration rate compared to the controls. It further indicates that control calves were more severely dehydrated during the course of the disease than those of ECO group; this is confirmed by the significant increase of serum Alb concentration only in Conts group and the significantly higher serum Alb concentration on D1PD recorded in this group.

The better hydration status of calves that received oregano essential oil denotes that the diarrhea was less severe in calves of this group, mitigating the fluid loss. This is partially confirmed by the significantly lower average fecal score in ECO group. Taking also into account that the duration of diarrhea course was similar between groups, it could be assumed that the severity of the intestinal mucosa damage in calves of ECO group might have been less severe compared to the controls. Such an effect cannot be ruled out since research findings from other animal species have shown that oregano essential oil has a protective effect on intestinal mucosa by modifying the intestinal microflora, inhibiting the expression of inflammatory cytokines and enhancing its antioxidative capacity[23,24].

The significant increase of serum K and P during diarrhea and the significantly higher values of these macroelements recorded in the control compared to the ECO group are attributed to the hypovolemia and the impaired renal function of the calves of this group. It is well documented that the increase in proximal tubular reabsorption of water and sodium along with the decreased glomerular filtration rate due to hypovolemia result in reduced distal tubular K secretion and, consequently, in increased K concentration in calves with prerenal azotemia[19]. Increased P concentration is usually observed in calves with hyperkalemia; it is mainly due to dehydration and prerenal azotemia but it is referred that acute acidemia might also be responsible for part of the increased P concentration[19].

The decrease in serum Ca concentration during diarrhea observed in the present study is in accordance with former reports[25,26] and is attributed to calcium loss with feces[17]. However, the decline was significant only in the control group; this finding probably indicates that diarrhea and, consequently, the fecal loss of Ca was more severe in this group.

Serum glucose and Na concentrations remained unaffected by both diarrhea and the administration of oregano essential oil. This is in contrast with other findings supporting that diarrheic calves have lower concentrations of glucose and Na[9,11,12] than the non-diarrheic ones. However, the calves finally included in this study had adequate milk consumption throughout diarrhea. In addition, all calves were offered an oral rehydrating solution that contained an energy source and Na twice a day. It seems that the applied management protected the diarrheic calves from hypoglycemia and hyponatremia.

The serum activity of AST was significantly lower after diarrhea compared to D2, and the activity of CK remained practically stable. This corresponds to the normal variation of these enzymes for calves of this age[27,28] and indicates that diarrhea is not associated either with liver or muscle damage. It further provides evidence that liver function is not impaired by the administration of oregano essential oil in diarrheic neonatal calves.

In the context of this study, the obtained results indicate that oral administration of oregano essential oil could prevent the impairment of the glomerular filtration rate of diarrheic calves and ameliorate the serum potassium, inorganic phosphorus and calcium imbalances.

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