



INVESTIGATION ON EFFECTS OF ORALLY GIVEN SEPIOLITE ON RUMINAL PROTOZOA IN CATTLE

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Abstract: Sepiolite has been used in antacid and antidiarrheal drugs for several years because of its adsorptive properties. Another important use of sepiolite is in drilling fluids. Sepiolite is used in drilling fluids, because the viscosity and gel strength of a sepiolite mud are not affected by variations in electrolytic content. It is suggested that sepiolite will be cause important effects on rumen microflora and fauna with its features. An important part of the digestion in the rumen that made up from microfauna created by rumen protozoa. In this study were evaluated the effect orally given sepiolite on rumen protozoa in totally 100 animals (80 experimental and 20 control group) ages between 6 and 18 months growth by public in Uşak Region. Besides the examination of rumen protozoa, all the animals were examined regarding to their clinical, haematological and biochemical parameters. It was envisaged that orally given sepiolite would increase the efficiency of the rumen protozoa and higher feed benefits in this study. The present study was considered to be scientifically assessed work of one of the most important values of our country sepiolite in animal health.

Keywords: Cattle, protozoa, rumen, sepiolite, Usak Province

Introduction: Sepiolite ($Mg_8Si_2O_{30}(OH)_4(OH_2)4H_2O$) is a natural clay mineral of polygorskite group which has an alkaline structure, and used either orally or topically because of its antioxidant, gastrointestinal protective, antibacterial (Almáida, 2013,

Delavarian *et al.*, 2013, Nezamzadeh-Ejhiéh and Kabiri-Samani, 2013). In Spain, 7% of total sepiolite was used in animal health and 4% in agriculture (Clarke, 1985). In Turkey, sepiolite were considered only as poultry in pets or poultry farms (Engel, 1991). It was reported that the presence of 1% sepiolite increased live weight gain and decreased serum cholesterol and triglyceride levels with relative abdominal fat weight (Sardi *et al.*, 2004; Tortuero *et al.*, 1992).

Antibiotic participation in animal feeds is prohibited in European countries, and this ban is

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widespread worldwide (Vondruskova *et al.*, 2010). Although sepiolite has various anti-bacterial effects without any adverse effects, most of antimicrobial drugs used in animal feeds have got adverse effects such as ischemic etiology (Hrenovic *et al.*, 2012; Elitok and Baser, 2016).

It has been shown that sepiolite can regulate microbial fermentation processes derived from the application of ruminants without improvement, improve the efficiency of microbial fermentation processes and prevent ruminal acidosis by preventing the multiplication of *S. bovis* (Serra *et al.*, 2013). In a study conducted in chickens (Mizrak *et al.*, 2013), it is reported that ascertainment of the elevation of fecal pH in animals with sepiolite added to their feeds supports a skepticism. Separately, sepiolite was reported to facilitate digestion by preventing agglomeration of feedstuffs (Alvarez, 1984; Castaing, 1989).

Compare to the untreated control group with sepiolite added to rations along with pine oil (Mcintosh *et al.*, 2003), the protozoa in the bovine rumen led to a reduction of 4.5% in the bacterial population, leading to a reduction in the level of rumen ammonia without association and regulation of digestion, leading to changes in VFA in the composition and in mating. Roger *et al.* (1990) and Meschy (1993) are other important studies reported to have facilitated digestion by causing cell wall breakdown of plant-containing cellulosic containing sepiolite. Structuring on the effects of sepiolite on the microbial populations of rumen and rumen digestion, which contains many elements such as calcium, in particular magnesium, is the most important minerals play a crucial role in providing the same energy and nitrogen in the environment (Ampian, 1984; Leonard and Martens 1996; Wylie *et al.*, 1985; Weiss, 2004; Smith, 1963). Similarly, Fonty *et al.* (1995) also found that Ca, Mg, Co including sepiolite affects the pH and rumen of the rumen of the minerals positively. However, Meshy (1993) reports that problems with cellulose degradation

can be overcome by rational calcium addition. Jouany and Morgavi (1994) report that clay minerals as sepiolite decrease methane, ammonia and carbon dioxide gases outside sourced by animals. Moreover, sepiolite reduces the number of bacteria and leads to lower methane production in the rumen (diminishing methane from ruminal fluid causes gratis energy loss for animals) increase in the amount of VFA's amount which the most important energy source of the cattle (Ivan *et al.*, 1992). Reduction of the methane gas from the medium means a decrease or an improvement in the population of methanogenic bacteria, i.e. an increase in the population of this bacteria and beneficial protozoa (Keeney, 1970; Wallace *et al.*, 2002).

It has been reported that toxicity of clay depends on the particle length of its fibers and the route of administration (Sohaebuddin *et al.*, 2010; Magdolenova *et al.*, 2014). It has been reported that sepiolite with a fiber length shorter than 6 µm does not cause cancer. The fiber length of Turkish sepiolite is shorter than 6 µm and carcinogenic effects are absent. Furthermore, oral administration of sepiolite is not leads to adverse effects, whereas inhalation, intrapleural or subperitoneal administration may cause (IARC, 1997).

The aim of this study was to firstly demonstrate how protozoa of rumen affected by adding sepiolite 2% amount of daily feed to the ration, and what kind of changes with clinical, hematological and blood biochemical findings.

Materials and Methods

Material: In this study, a total of 100 cattle (control n = 20, n = 80) aged between 6 and 18 months in Uşak Province were used. Sepiolite was administered orally in a weight 2% of the total amount of food given daily to the animal ration for 3 days in study group. Nothing was add to feeds of control group animals. Clinical, rumen fluid, haematological and serum biochemical measurements were detected in all the animals in days of 1, 2 and 3 by beginning of the trial. This study has been carried out under

the Ethics Committee of the Afyon Kocatepe University (AKUHADYEK) with the reference number 490-15 and was supported by Afyon Kocatepe University Scientific Research Projects Coordination Unit (BAPK) with the reference number 16. SAGBIL.14

Routine Clinical Experiments: Clinical examinations such as body temperature, respiration and heart rate, number of rumen contractions in 5 minutes were performed according to the method of reported by Hungate (1966).

Hematological Examinations: Blood samples were taken from V. jugularis in all the animals and hematological examinations such as erythrocyte, total leukocyte, hematocrit, hemoglobin, MCV, MHC, and MCHV were measured using commercial test kits via blood cell counter (Mindray BC2800 Vet).

Serum Biochemical Tests: Serum AST, LDH, total protein (TP), albumin (ALB), glucose (GLU), urea (UREA) and creatinine (CREA) were measured by autoanalyser (Roche brand Cobas C111 Model) using commercial test kits.

Rumen Fluid Examinations: Methylene blue test, total infusoria count and sedimentation test along with rumen fluid pH (Mulrictix 10 SG-

Bayer®-Germany) were measured in fresh rumen contents taken from rumen by tube methods reported by Boyne *et al.* (1957). Ruminal ammonia is measured according to the method reported by Inal (1991).

Statistical Analysis: Statistical calculations of the groups were made according to the ANOVA method. The Duncan test was used to determine the difference between groups in this study using SPSS 18.0 (Inc., Chicago, Ill., USA) package program for Windows. Data were presented as mean ± standard error and p <0.05 was considered significant.

Results: Fifty of 80 animals assigned as the study group were females, remaining 30 were males, and the average age of the study group was 14.2 months. Fourteen of the 20 cattle in the control group were female and 16 were male. The mean age was 14.0 months and there was no statistically significant difference between two groups in terms of age (p > 0.05).

Clinical Examination Findings: There was no significant difference between the control group and the study group in terms of body temperature, respiration and heart rate and ruminal movements at 5 minutes (p > 0.05) (Table 1).

Table 1. Comparison of body temperature, heart and respiratory frequencies and ruminal movements of the animals used in the study

Groups/Parameters	Control	1st Day	2nd Day	3rd Day	Reference values*	P
Temperature (°C)	38.44± 3.26	38.46 ± 3.04	38.50 ± 2.88	38.12 ± 2.54	37.5-38.6	>0.05
Pulse (rates/min)	87±2	87±1	87 ±3	87 ±0	70-120	>0.05
Respiration (rates/min)	24±2	25±0	24±3	25±1	15-30	>0.05
Rumen movement (contraction/5 min)	9± 2	9± 2	9 ± 1	9± 3	6-12	>0.05

^{a,b,c} Different letters within the same column indicate statically significant differences between the mean values of the groups (p <0.05). *İmren (1997).

Ruminal Fluid Findings: No differences were detected regarding rumen pH between the groups. However, it was observed that the mean number of infusoria and sedimentation time

higher than that those of the control group. On the contrary, means of methylen blue and ammonia significantly (p <0.05) lower than those of the control group (Table 2).

Table 2. Statistical analyses of ruminal fluid in control and study groups.

Groups	pH	Infusoria (mm³)	Methylen Blue Test (min)	Sedimentation Test (min)	Rumen fluid ammonia/ (mg/L)
	X±SD	X±SD	X±SD	X±SD	X±SD
Control	7.20±0.00	182.40±42.10 ^c	3.86±0.30 ^a	4.46±0.60 ^b	18.43±1.38 ^a
1st Day	7.10±0.00	252.78±28,10 ^b	3.10±0.20 ^b	5.30±0.40 ^a	16.06±1.30 ^b
2nd Day	7.20±0.00	246.54 ±32,76 ^b	3.08±0.40 ^b	5.28±0.50 ^a	15.68±1.22 ^b
3rd Day	7.20±0.00	369.30±40,40 ^a	2.42±0.30 ^c	5.30±0.60 ^a	15.20±1.32 ^b
P	p>0.05	p<0.05	p<0.05	p<0.05	p<0.05

^{a,b,c} Different letters within the same column indicate statically significant differences between the mean values of the groups (p <0.05)

Hematological Findings: According to the data obtained from the measurements shown that means of RBC count in the study group were found to be significantly higher than that of the control group (3.30 ± 1.00) on the 3rd day, although within the reference limits. Conversely, there were no significant differences ($p > 0.05$) between the control group and the study group regarding to in terms of numerical WBC mean. it was observed that On the third day of the mean level of HGB (12.40 ± 2.30) significantly higher ($p < 0.05$) than that of the control group (9.96 ± 1.24), though within the reference limits. The study group means of HTC was found to be significantly higher ($p < 0.05$) than the mean of the control group (26.40 ± 1.20), although within the reference limits. The lowest mean of MCV (40.98 ± 2.20) was obtained on day 3 of the study group. The highest MCHC levels were obtained in the control group (37.02 ± 3.40) and on the third day (37.68 ± 4.12) in the study group.

Metabolic profile findings: The mean values of AST and LDH enzyme levels in this study and control groups were within the reference limits and no statistically significant difference was found between the groups ($p > 0.05$). Means of UREA and CREA levels in the control group (51.48 ± 3.64 ; 1.74 ± 0.26 respectively) were higher than those of the study group and difference was significantly higher ($p < 0.05$), although all the levels in both groups were found to be within the reference limits. As opposed to this condition; the mean TP and ALB levels in the study group were significantly higher than those of the control group (6.38 ± 1.16 ; 3.02 ± 0.14 respectively) and difference was statically important ($p < 0.05$) (Table 4).

Discussion: Recently, alternative feed additives such as probiotics (bacteria, fungi and yeast), prebiotics and enzymes have been used

frequently in animals to increase health, efficiency and performance (Görgülü *et al.*, 2003, Kantautaitė *et al.*, 2006, 2009; Fratter, 2014). Unfortunately, the use of these substances has been accompanied by the emergence of many problems on farms (such as mad cow antibiotic resistance, milk freshening, pollution) (Jounay and Morgavi, 2007; EFSA, 2013; Rodríguez-Beltrán *et al.*, 2013).

With the detection of the adverse effects of the residue of these products on the environment and human health, scientists have come up with alternative products that will increase animal yields, which are not harmful to environment and human health (EFSA 2013, Vondrovská *et al.*, 2010, Mandal *et al.*, 2014).

Clay is a natural additive that has been included in the diet of pets in recent years. Some investigations have shown that the nutritional properties of clays (antibacterial and detoxification properties) in livestock production (Ouhida *et al.*, 2000a; Ouhida *et al.*, 2000b, Xia *et al.*, 2005) as a digestive facilitators (Heillin and Murray, 1994; Rodríguez-Beltrán 2013) (Xia *et al.*, 2005, Kaboul and Ouachem, 2012).

Sepiolite is natural clay with high toxin absorption capacity, antibacterial activity and rich mineral content (Mg, Cu, Al, Ca etc.) along with no harmful effect on human health and environment (Galan and Castillo, 1984, Clarke, 1985; Clarke, 1989; Muirhead, 1998, EFSA 2013).

In this study, we did not find sepiolite did not cause any negative change in clinical findings caused by sepiolite as an additive ingredient as reported before by Mandal *et al.*, 2014, Mizrak *et al.*, 2013, Nguyen *et al.*, 2008, EFSA, 2013. Moreover no significant difference was observed in the T, P and R frequencies between the control group and the study group animals in terms of RH at 5 minutes in our study.

Table 3. Statistical analyses of hematological findings in control and study groups

Groups	WBC (10 ³ /μL)	RBC (10 ⁶ /μL)	HGB (g/dL)	HCT (%)	MCV (fl)	MCHC (g/dL)	MCH (pg)	LENF (10 ³ /μL)	MONO (10 ³ /μL)	NOTR (10 ³ /μL)	EOS (10 ³ /μL)	BAS (10 ³ /μL)
	X±SD	X±SD	X±SD	X±SD	X±SD	X±SD	X±SD	X±SD	X±SD	X±SD	X±SD	X±SD
Control	9.34± 1.88	6.30± 1.00 ^b	9.96± 1.24 ^b	26.40±1.20 ^b	42.02 ± 2.40 ^b	37.02 ± 3.40 ^a	15.60± 1.86 ^b	5.20± 0.60 ^a	0,94± 0,04	3.60± 0.60 ^b	0.70± 0.20 ^b	
1st Day	9.20± 1.60	6.20± 1.20 ^b	10.08± 1.22 ^b	30.60± 3,20 ^a	49.34± 2.22 ^a	32.86 ± 3.28 ^b	16.10±2.68 ^a	4.22± 0.40 ^b	0,98± 0,00	4.68 ± 0.46 ^a	1,30± 0.30 ^a	-
2nd Day	9.16± 1.44	6.36± 1.12 ^b	10.04± 1.20 ^b	31.00± 4,30 ^a	48.96± 2.44 ^a	32.68± 3,20 ^b	15.98± 2,04 ^a	4.24± 0.30 ^b	0,96± 0,02	4.32± 0.38 ^a	1.26± 0.36 ^a	-
3rd Day	9.20 ± 1.32	7.40± 2.30 ^a	12.40± 2.30 ^a	30.30± 4,20 ^a	40.98±2,20 ^c	37.68± 4.12 ^a	16.20± 2.60 ^a	4,30± 0.28 ^b	0,94± 0,03	4.28± 0.40 ^a	1,24± 0.28 ^a	R
Ref. Values*	4-12	5-10	8-15	24-46	40-60	30-60	11-17	3-7.5	0.0-0.9	0.6-4	0-2.4	0-2
P	> 0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	>0,05	>0,05	<0,05	<0.05	>0.05

^{a,b,c} Different letters within the same column indicate statically significant differences between the mean values of the groups (p <0.05).

R: Rarely. * Filder (2016).

Table 4. Comparison of the statistics of the mean of the metabolic profile parameters

Groups	AST (IU/L)	LDH (IU/L)	UREA (mg/dL)	CREA (mg/dL)	TP (g/dL)	ALB (g/dL)	GLU (mg/dL)
	X±SD	X±SD	X±SD	X±SD	X±SD	X±SD	X±SD
Control	96.46±8,24	1138.34±86.30	51.48±3.64 ^a	1.74±0.26 ^a	6.38±1.14 ^b	3.02±0.16 ^b	5.84±1.62
1st Day	98.03±9.64	1140.40±78.42	39.04±4.02 ^b	1.01±0.10 ^b	7.80±1.28 ^{ab}	3.94±0.38 ^a	5.68±1.44
2nd Day	97.56±8.42	1136.28±92.64	38.96 ±3.72 ^b	1.14±0.16 ^b	7.78±1,00 ^a	3.92 ±0,38 ^a	5.72±1.56
3rd Day	96.64±8.02	1138.76±90.08	38.76±4.28 ^b	1.06±0.12 ^b	7.84±1,30 ^a	3.96 ±0,26 ^a	5.80±1.70
Ref. Values*	<240	<1500	42.8-64.2	1-2	6.7-7.5	3.0-3.5	4.5-7.5
P	>0.05	>0.05	<0.05	<0.05	<0.05	<0.05	>0.05

^{a,b,c} Different letters within the same column indicate statically significant differences between the mean values of the groups (p <0.05). * Altıntaş ve Fidancı (1993).

It was determined that the study group with sepiolite added to rations led to a better healing of rumen in optimal conditions in animals in the present study. There was no statistically significant difference between the rumen pH levels of the study group and that of the control group, but the ammonia levels of rumen were lower in the study group. In a study conducted in chickens (Mizrak *et al.*, 2013), it was found that faecal pH was elevated in animals whose sepiolite was added to their feed. Wallace *et al.* (2002) and McIntosh *et al.* (2003) reported that the reduction in NH₃ levels was desirable for animal health, as amino acid deamination prevented the reduction of ruminal microorganisms. As a matter of fact, the decrease in ammonia level is very important to prevent nitrogen loss in the form of NH₃ in ruminants, to increase utilization of energy and nitrogen in feeds, and also to prevent environmental pollution by reducing atmospheric CH₄ and NH₃ gas emissions (Tamminga, 1996; Greathead, 2003).

We did not find a literature review on the direct effects of sepiolite on the ruminant digestive system in our extensive literature review. However, other studies related with sepiolite in ruminants offer important ideas. In a study (Serra *et al.*, 2013), naringin, a mixture of bitter orange extract and sepiolite, regulates microbial fermentation processes derived from the application of concentrated rations in ruminants, increases the efficiency of microbial fermentation processes and prevents ruminal acidosis by inhibiting the proliferation of *S. bovis*. It has also been reported that sepiolite added to bait will facilitate digestion by inhibiting the agglomeration of feedstuffs (Rodrigues-Beltran *et al.*, 2013; Parisini *et al.*, 1999).

Jouany and Morgavi (2007) reported that expect of sepiolite, some clays did not cause decreased methane, ammonia and carbon dioxide gases, while cause increased bacterial counts and decrease the number of protozoa in the rumen. These researchers also noticed that sepiolite did

not cause to decrease in protozoan counting rumen. However, in our study similarly observed an increase in the total number of protozoa.

Although we did not measure the reactive oxygen capacity in cattle in this study, Amati *et al.* (1997) reported that sepiolite could that along with increased phagocytic cells counts, even though not as much as kaolin. Similarly findings were determined in the study group animals with regarding to neutrophil counts when compared to the control group's animals. Moreover, in our study, sepiolite given orally did not lead to an increase inflammatory process in animals as leukocytes. In a study conducted in poultry (Safamehere, 2008) shown that hematological performance was better in animals which fed sepiolite. In another study with birds (Ibrahim *et al.*, 2000), hematologic parameters were found to improve in birds with Newcastle disease, when clay mineral was added to their feed.

In this study, it was determined that the animals which sepiolite added to their rations did not changes AST, LDH and GLU levels, but decreased UREA and CREA levels, and increased TP and ALB levels. AST and LDH enzymes are elevated enzymes in the damage of organs and tissues such as liver, kidney, muscle (Giannini *et al.*, 2005). In our study, the absence of increases in the levels of these enzymes is accepted as a prove of no toxic effects of sepiolite on organs such as the liver, kidney and heart, and it did not lead to an increase in the number of ruminal microorganisms, such as bacteria, which can lead to toxicity. As a matter of fact, sepiolite has been reported to have a higher effect of absorbing toxins and preventing cell damage than many other clay minerals (Weaver *et al.*, 2013). UREA and CREA were found to be a good indicator of renal damage (Alexopoulos *et al.*, 2007). In this study, UREA and CREA levels of the study group were found to be lower than that of the control group. This can be interpreted as the absence of sepiolite given to kidney damage. Moreover, in another

study (Yalçın *et al.*, 2016) in which sepiolite was added to chickens' feed; sepiolite had been reported to cause an increase in serum TP levels. Similar findings were obtained in our study.

Consequently, it has been determined that sepiolite is not a negative effect on rumen microfauna and blood parameters when given by oral route and it is a natural clay mineral which causes positive changes in some parameters such as rumen fluid composition and TP. Thus, we advise that sepiolite can be safely used in animal health.

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