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Effect of subacute ruminal acidosis (SARA) on ruminal fluid and faecal compositionsin dairy cows

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Abstract: *The objectives of this study was to diagnose and study the effects of subacute ruminal acidosis (SARA) on ruminal fluid and fecal compositions in dairy cows.SARA was diagnosed by measuring pH of rumen fluid collected by rumenocentesis four hours after feeding. Out of 100 dairy cows examined for SARA based on rumen fluid pH, it was noticed that 20 dairy cows had rumen fluid pH ranging from 5.2 to 5.6 for more than 180 minutes/day and were considered as positive for SARA and 80 cows had ruminal fluid pH more than 6.2 and were considered as negative for SARA.*
In the present study 20 SARA affected dairy cows compared with 20 not suffering from SARA (normal). Rumen fluid examination indicated significantly lower (P≤0.05) sedimentation activity time (SAT), significantly increased methylene blue reduction time (MBRT) and poor to moderate iodophilic activity in SARA affected dairy cows. Mean total protozoal count in SARA affected dairy cows was statistically low (P≤0.05) and there was a shift in the rumen microbial composition to Gram-positive bacteria in SARA affected dairy cows. The fecal pH in SARA affected dairy cows was significantly low. SARA affected fecal consistency and fecal structure in dairy cows.
Key words: *Subacute ruminal acidosis, Dairy cow, Rumen fluid pH, Rumen protozoa, Rumenocentesis*

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I. Introduction

Among different diseases affecting the forestomach of dairy cows, SARA is a common and economically important problem in dairy herds affecting fermentation in rumen, productivity and farm profit. It is estimated that the economic loss associated with SARA is US $ 500 million to US $ 1 billion annually. The losses are mainly the result of reduced milk production, premature culling and mortality (Enemark, 2008). SARA is the consequence of feeding high grain diet to dairy cows which are previously adapted to digesting predominantly forage diets and characterized by daily episodes of low rumen fluid pH.Diagnosis of SARA in a dairy cow based only on clinical signs is very difficult and studying rumen fluid pH is the only method recommended for diagnosis of SARA in dairy cows (Tajik and Nazifi, 2011) though few other parameters such as estimation of lipopolysaccharide in rumen and rumen temperature are tested by earlier workers (Khafipour et al., 2009a). In India, not much research work has been carried out on the alterations caused by SARA. Thus a study was conducted to determine the alterations associated with SARA in dairy cows.

II. Material and Methods

Experimental Animals: Dairy cows placed at dairy farm, Department of ILFC, Veterinary College, Hebbal, Bangalore and other organized dairy farms in and around Bangalore served as experimental animals in this study. A total of 100 dairy cows were selected randomly for this study. Rumen fluid was collected from dairy cows by rumenocentesis as explained by Garrett et al. (1999). Rumen fluid was collected 4 hours after feeding the dairy cows.

Rumen FluidAnalysis: The pH of rumen fluid was determined using portable digital pH meter immediately after collecting of the rumen fluid. Dairy cows with rumen pH ranging from 5.2 to 5.6 were selected and cases where rumen pH continues to be in the range of 5.2 to 5.6 for minimum of 3 hours were considered as positive for SARA (Gozho et al., 2005). Dairy cows with ruminal pH above 5.6 were considered as negative for SARA and dairy cows with ruminal pH less than 5.2 were considered to be positive for acute ruminal acidosis. Twenty dairy cows suffering from SARA and twenty dairy cows not suffering from SARA (normal) were employed.

Rumen fluid collected from dairy cows was subjected for estimation of SAT, MBRT, total protozoal count, iodophilic activity and microbial composition. For measuring SAT, Nicholas and Penn(1958) and for

measuring of MBRT Dirksen(1969)methodswere adapted. The iodophilic activity was graded as nil (-), poor (+), moderate (++), good (+++), and very good (++++), depending on the quantity of starch contained as described by Rosenberger (1979). A modified method of Warner (1962) described by Sankaranarayanan and Nambiar (1972) was followed for enumeration of total protozoal count(10^5). For microbial composition; Rumen fluid smear was prepared over a glass slide and stained with Gram's stain and observed under oil immersion of microscope. Observation was recorded as Gram-positive and Gram-negative organisms.

Fecal Sample Assay: Fecal pH was estimated immediately after being voided using narrow range pH paper and faecal consistency was classified as loose, well-formed and firm based on physical observation of faecal sample immediately after it is being voided, as per the method explained by Kleen et al. (2003). Faecal structure was classified as coarse where large particles of fiber, undigested grains or fibrin clots were seen and classified as fine where no large particles of fiber, undigested grain or fibrin clot were observed.

Statistical Analysis

Statistical analysis was performed using the statistical software GraphPad Prism, version 5 for windows. The data were analyzed by Student's t-test (One-sample, paired and unpaired) to arrive at conclusion.

III. Results

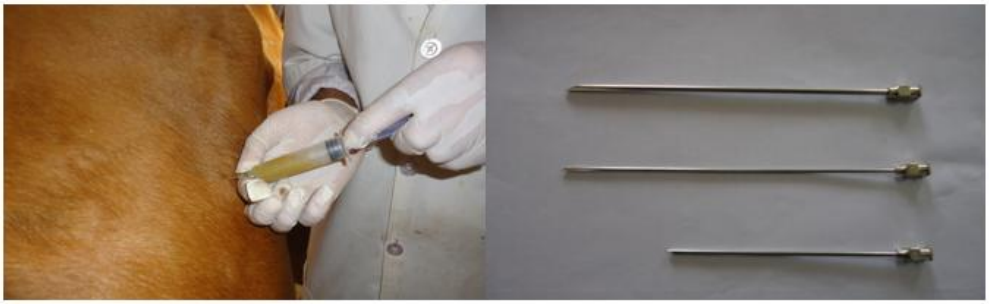

Plate 1: Collection of rumen fluid by rumenocentesis using 100-120 mm long stainless steel needles

Table 1: Alterations in rumen fluid (pH, SAT, MBRT and protozoal count) in normal and SARA affected dairy cows

S/No	Rumen Fluid pH		Sedimentation Activity Time (min)		Methylene Blue Reduction Time (min)		Protozoal Count (10^5)	
	Normal	SARA	Normal	SARA	Normal	SARA	Normal	SARA
1	6.5	5.5	4.0	2.0	2.0	3.0	0.98×10^5	0.2×10^5
2	6.3	5.5	4.5	1.5	1.5	2.5	0.96×10^5	0.32×10^5
3	6.4	5.4	4.0	2.0	2.5	3.0	1.1×10^5	0.4×10^5
4	6.7	5.4	5.0	2.0	2.0	3.0	0.90×10^5	0.38×10^5
5	6.5	5.4	4.0	3.5	2.0	3.0	0.93×10^5	0.3×10^5
6	6.4	5.4	5.0	3.0	3.0	3.0	1.2×10^5	0.41×10^5
7	6.5	5.5	5.0	2.0	2.0	3.0	1×10^5	0.42×10^5
8	6.5	5.5	7.0	2.0	2.5	4.0	0.79×10^5	0.38×10^5
9	6.6	5.6	6.0	2.5	3.0	3.0	0.875×10^5	0.48×10^5
10	6.3	5.6	5.0	2.0	2.0	2.8	0.95×10^5	0.8×10^5
11	6.6	5.6	8.0	3.0	2.5	3.0	1.5×10^5	0.61×10^5
12	6.6	5.6	5.0	3.0	2.0	4.0	0.87×10^5	0.35×10^5
13	6.4	5.6	7.0	3.5	4.0	2.5	1.2×10^5	0.68×10^5
14	6.6	5.6	7.0	2.5	3.0	3.0	1.3×10^5	0.45×10^5
15	6.5	5.5	8.0	3.0	3.0	3.0	1.62×10^5	0.35×10^5
16	6.5	5.5	4.0	2.5	2.0	3.0	1.5×10^5	0.45×10^5
17	6.4	5.4	6.0	2.0	3.0	2.5	1.3×10^5	0.31×10^5
18	6.4	5.4	7.0	2.0	4.0	2.5	1.5×10^5	0.51×10^5
19	6.4	5.4	7.5	3.0	3.0	4.0	1.2×10^5	0.5×10^5
20	6.5	5.5	7.8	3.0	3.0	4.0	2.1×10^5	0.65×10^5
Mean ± SE	6.49 ±0.02[a]	5.50 ± 0.01[b]	5.84 ± 0.32[a]	2.50 ± 0.13[b]	2.60 ± 0.15[a]	3.09 ± 0.11[b]	$1.89 \pm 0.07 \times 10^{5a}$	$0.44 \pm 0.03 \times 10^{5b}$

Note: Means bearing different superscript differ significantly ($P \leq 0.05$)

Rumen fluid was collected by rumenocentesis from individual dairy cows four hours after feeding and adequate quantity of rumen fluid could be collected by this method(Plate 1). Dairy cows having rumen fluid pH in the range of 5.2 to 5.6 for a period of minimum three hours were considered as positive for Sub Acute Rumen Acidosis.Out of 100 dairy cows tested, 20 dairy cows had ruminal fluid pH ranging between 5.2-5.6 and remaining 80 cows had ruminal fluid pH more than 6.2 which indicated the

Table 2a: Iodophilic activity and microbial composition of rumen fluid in normal and SARA affected dairy cows

S/ No	Iodophilic Activity		Predominant Microbial Composition	
	Normal	SARA	Normal	SARA
1	Moderate (++)	Poor (+)	Gram positive	Gram positive
2	Moderate (++)	Poor (+)	Gram negative	Gram positive
3	Moderate (++)	Moderate (++)	Gram positive	Gram negative
4	Moderate (++)	Poor (+)	Gram negative	Gram positive
5	Moderate (++)	Moderate (++)	Gram positive	Gram positive
6	Good (+++)	Moderate (++)	Gram negative	Gram positive
7	Moderate (++)	Poor (+)	Gram negative	Gram positive
8	Moderate (++)	Moderate (++)	Gram positive	Gram positive
9	Moderate (++)	Poor (+)	Gram negative	Gram negative
10	Moderate (++)	Moderate (++)	Gram negative	Gram positive
11	Good (+++)	Moderate (++)	Gram negative	Gram positive
12	Good (+++)	Moderate (++)	Gram negative	Gram positive
13	Good (+++)	Moderate (++)	Gram negative	Gram positive
14	Moderate (++)	Poor (+)	Gram negative	Gram positive
15	Moderate (++)	Moderate (++)	Gram negative	Gram positive
16	Moderate (++)	Poor (+)	Gram negative	Gram positive
17	Moderate (++)	Poor (+)	Gram negative	Gram positive
18	Good (+++)	Poor (+)	Gram negative	Gram positive
19	Moderate (++)	Poor (+)	Gram negative	Gram positive
20	Good (+++)	Poor (+)	Gram negative	Gram positive

Table 2b: Summary of iodophilic activity and microbial composition of rumen fluid in normal and SARA affected dairy cows

Parameters		Normal		SARA	
		No. of cows	Percent (%)	No. of cows	Percent (%)
Iodophilic activity	Poor (+)	0	0.0	11	55
	Moderate (++)	14	70	9	45
	Good (+++)	6	30	0	0.0
	Total	20	100	20	100
Microbial composition	Gram-negative	16	80	2	10
	Gram-positive	4	20	18	90
	Total	20	100	20	100

prevalence of SARA was 20 percent in dairy cows.The mean ± SE SAT in normal and SARA affected dairy cows was 5.84 ± 0.32 and 2.50 ± 0.13respectively, and there was a significant ($P \leq 0.05$) difference between two values indicating that SAT was statistically low in

Table 3a: Fecal pH, consistency and structure in normal and SARA affected dairy cow

S/No	Fecal pH		Fecal Consistency		Fecal Structure	
	Normal	SARA	Normal	SARA	Normal	SARA
1	6.7	5.9	Well formed	Loose	Fine particle	Coarse
2	6.2	6.0	Well formed	Well formed	Fine particle	Coarse
3	6.0	5.9	Loose	Loose	Coarse	Coarse
4	6.5	6.0	Well formed	Loose	Fine particle	Fine particle
5	6.5	6.0	Well formed	Loose	Fine particle	Coarse
6	6.5	6.3	Well formed	Loose	Fine particle	Coarse
7	6.2	6.0	Firm	Loose	Fine particle	Coarse
8	6.5	6.0	Firm	Loose	Coarse	Coarse
9	7.8	5.9	Well formed	Loose	Fine particle	Coarse
10	7.2	6.1	Firm	Loose	Fine particle	Coarse
11	7.0	6.4	Well formed	Loose	Fine particle	Coarse
12	7.0	5.8	Well formed	Loose	Coarse	Fine particle
13	7.0	6.7	Well formed	Loose	Fine particle	Fine particle
14	6.5	6.8	Well formed	Loose	Fine particle	Coarse
15	7.2	6.2	Loose	Loose	Fine particle	Coarse
16	7.0	6.0	Well formed	Firm	Fine particle	Coarse
17	7.4	6.5	Well formed	Well formed	Fine particle	Coarse
18	7.0	6.0	Well formed	Loose	Fine particle	Coarse
19	7.0	6.0	Firm	Loose	Coarse	Coarse
20	7.0	6.2	Well formed	Loose	Fine particle	Coarse
Mean ± SE	6.81 ± 0.10^{a}	6.14 ± 0.06^{b}				

Note: Means bearing different superscript differ significantly ($P \leq 0.05$)

SARA affected dairy cows (Table 1).It was observed that 9 (45%) and 11 (55%)dairy cows suffering from SARA had moderate and poor iodophilic activities respectively. In contrast 6 (30%) and 14 (70%) of normal dairy cows exhibited good and moderate iodophilic activity, and It was observed that 90 percent of rumen fluid collected from dairy cows suffering from SARA had Gram-positive bacteria whereas 80 percent rumen fluid collected from normal cows had Gram-negative bacteria (Table 2a and 2b).Fecal pH in SARA affected dairy cows was significantly ($P \leq 0.05$) lower as compared to normal cows and fecal consistency was affected in SARA affected dairy cows and consistency tend to be loose in SARA affected dairy cows. This study indicated that fecal structure was affected in SARA affected dairy cows and was coarse in majority of dairy cows (Table 3a and 3b).

IV. Discussion

Table 3b: Summery of fecal consistency and structure in normal and SARA affected dairy cows

Parameters		Normal		SARA	
		No. of cows	Percent (%)	No. of cows	Percent (%)
Fecal consistency	Well formed	14	70	2	10
	Loose	2	10	17	85
	Firm	4	20	1	5
	Total	20	100	20	100
Fecal structure	Fine particles	16	80	3	15
	Coarse	4	20	17	85
	Total	20	100	20	100

and adequate quantity of rumen fluid was collected by this method. Rumenocentesis is considered as a better field test in comparison to the oro-ruminal probe for the measurement of rumen pH (Duffield et al., 2004). Hence it is safe to conclude that rumenocentesis is a suitable method for collecting rumen fluid in dairy cows

and rumen fluid was collected 4 hours after feeding because the time of sampling after feeding is important. It is suggested that the rumen fluid sample should be collected two to four hours after feeding the animal (Nordlund et al., 1995) and (Kleen et al., 2003). Other methods of rumen fluid collection namely indwelling electrode and ruminal cannulation have got their own limitations such as contamination and clogging of the electrode (Enemark et al., 2003) and disturbance of the animal (Nocek, 1997). A portable pH meter was utilized in the study to measure rumen fluid pH and it is considered suitable to measure pH of rumen fluid and yields very similar pH reading as did a standard meter with a pH probe (Garrett et al., 1999). Hence it can be concluded that pH of rumen fluid can be measured using portable pH meter. In the present study dairy cows suffering from SARA were identified by evaluating rumen fluid pH. Rumen fluid was collected by rumenocentesis and dairy cows having rumen fluid pH in the range of 5.2 to 5.6 for a period of minimum three hours were considered positive for SARA.This rumen fluid pH range of 5.2 to 5.6 for definition of SARA is as per the observation made by Gozho et al. (2005) who have defined SARA as daily episodes of low rumen pH between 5.2 to 5.6 for at least 180 min/day. Rumen pH below 5.6 for a duration of minimum of 180 min/day is required to activate an inflammatory response during SARA (Gozho et al., 2005). Diagnosis of SARA based on the free rumen LPS concentration is suggested by Gozho et al. (2005). However, this method seems to be more cumbersome. Similarly, rumen fluid temperature, faecal lipopolysaccharide and change in rumen microflora and composition have been used by few workers to diagnose SARA in dairy cows (Nagaraja et al., 1978, Goad et al., 1998, Al-Zahal et al., 2008 and Gakhar et al.,2008).However, at present these diagnostic approaches are for research purpose and yet to reach the level of field application.The incidence of SARA in this study was 20 per cent. This observation agrees with the finding of Garret et al. (1999)and Oetzel et al. (1999) who have reported prevalence of SARA to be 20 to 30 per cent. However,Bramley et al. (2008) and O'Grady et al. (2008) observed SARA in 10 per cent of the dairy animals. This difference may be attributed to difference in geographical area, type of feed and ruminal environment in term of flora and ruminal mucosa as rightly reported by Kleen et al. (2003). The Mean ± SE sedimentation activity time of normal cows was 5.84 ± 0.32 and the same in SARA affected dairy cows was 2.50 ± 0.13. The Mean SAT was statistically ($P \leq 0.05$) low in cows suffering from SARA. In healthy cattle SAT varies from 4-8 min depending on the ration and time after feeding (Rosenberger, 1979). These observation agrees with the findings of Rosenberger (1979) who stated that SAT may be rapid in cases of decreased ruminal pH. The rapid sedimentation activity of rumen fluid in SARA affected dairy cows may be due to decreased rumen pH, starvation or decreased dry matter intake observed in SARA. The Mean ± SE methylene blue reduction time of rumen fluid from dairy cows suffering from SARA was 3.09 ± 0.11 and it ranged from 2.5-4 minutes. Statistically significant ($P \leq 0.05$) difference was recorded when mean values of MBRT of SARA affected dairy cows were compared with that of normal dairy cows. This study indicated that MBRT was statistically high in SARA affected dairy cows. This observation agrees with the findings of Rosenberger (1979) and Gnanaprakasam (1988). Themethylene blue reduction time increases whenever rumen pH decreases and MBRT will be considerably get delayed when the rumen fluid pH is less than 5.0 (Rosenberger, 1979 andGnanaprakasam, 1988). Hence it may be safe to conclude that MBRT will be increased in SARA affected dairy cows where rumen fluid pH decreases from physiological level to as low as 5.3. Further, MBRT can be increased whenever the animal is starved (Steen, 2001). In cases of SARA, the dry matter intake will be reduced and appetite of the animal will be erratic. This may be another reason for observation of increased MBRT in SARA affected dairy cows. The Mean ± SE total protozoal count in SARA affected dairy cows was 0.44 ± 0.3 as against 1.89 ± 0.07 in unaffected dairy cows. There was a statistically significant ($P \leq 0.05$) difference in the mean total protozoal count between SARA affected dairy cows and normal dairy cows indicating total protozoal count was statistically low in SARA affected dairy cows. This observation is in agreement with the finding of Belknap and Navarre (2000) who reported that a fall in the pH of rumen contents is generally accompanied by a decrease in the protozoal concentrations. The large entodiniomorphs are the most sensitive of the protozoal species, whereas the trichostomatids are the most tolerant to low pH. Nearly all protozoa die when pH declines to 5.0. Goad et al. (1998) concluded that a decline in the concentration of ciliated protozoa may be the only microbial indicator of SARA. The decline in protozoal count in SARA affected dairy cows may also have attributed to reduce DMI as underfeeding also cause a reduction in protozoal concentration (Dirksen, 1990). Other factors which could influence protozoal count include rate of feed consumption, rate of food passage and salivary production (Franzolin and Dehority, 1996). The reduction in mean protozoal count in SARA affected animals could be attributed to reduced pH of rumen content, reduced DMI, rate of feed consumption and rate of passage of food. In this study it was observed that iodophilic activity of rumen fluid collected from SARA affected dairy cows was moderate in 45 per cent of cases and poor in 55 per cent of cases. On the other hand, iodophilic activity in normal dairy cows was good in 30 per cent of animals and moderate in 70 per cent of dairy cows. This may be attributed to significantly reduced total rumen fluid protozoal count noticed in the present study. Further, the degree of reduction in total protozoal count varies with different species of rumen protozoa and the degree of iodophilic activity of

different species of protozoa also varies (Goad et al., 1998 and Belknap and Navarre, 2000).This may be the reason for observation of reduced iodophilic activity of rumen fluid collected from SARA affected dairy cows. In the present study it was noticed that there was a shift in the ruminal bacterial composition in SARA affected dairy cows and Gram-positive bacteria was predominantly seen in SARA affected dairy cows. This observation is in agreement with the finding of Nagaraja et al. (1978), Goad et al. (1998),Khafipour et al. (2009a) and Colman et al. (2010). The microbial composition of rumen will be different between SARA affected and non-affected cows, between mild and severe induced SARA and between grain induced and alfalfa pellet induced SARA (Khafipour et al., 2009a). In general, rumen acidity results in a reduction in cellulolytic bacteria and a shift inbacterial population so that Gram-positive cocci and rods predominant (Nagaraja et al., 1978 and Goad et al., 1998). Gram-negative organisms are more in the ruminal fluid of normal cows (Rosenberger, 1979and Srinivasan, 1991). As the ruminal pH decreases it leads to selective destruction of Gram-negative bacteria and favors proliferation of Gram-positive bacteria (Elam, 1976). This may be the reason for observation of predominantly Gram-positive bacteria in the rumen fluid collected from SARA affected dairy cows. The Mean ± SE fecal pH in SARA affected dairy cows was 6.14 ± 0.06 and the fecal pH in normal dairy cows was 6.81 ± 0.01. It was also noticed that fecal pH in SARA affected dairy cows was significantly ($P \leq 0.05$) lower. Similar observations have been made by Kleen et al. (2003) andGrove-White (2004) who have reported that the pH of feces in SARA affected dairy cows was lower than the unaffected dairy cows. However,Gakhar et al. (2008) reported that experimental SARA induction had no effect on fecal pH. Nordlund (2004) believed that fecal pH is an indicator of small intestinal pH, but not necessarily ruminal pH. However, it may be safe to conclude that reduced rumen pH might have influenced fecal pH in the present study. In the present study it was noticed that fecal consistency in SARA affected dairy cows was loose in 85 per cent of cases as against 10 per cent in normal animals indicating that fecal consistency tends to be loose in SARA affected dairy cows. Similar observations have been made by Nocek (1997), Carter and Grovun (1990) Kleen et al. (2003), Oetzel (2003) and Nordlund (2004). In cases of SARA there will be increase in osmolality of the rumen fluid which draws fluid into the rumen leading to diarrhea in SARA affected dairy cows (Carter and Grovun, 1990). Another reason for diarrhea observed in cases of SARA affected dairy cows may be due to extensive hindgut fermentation as indicated by Huber (1976) and Nordlund (2004).Fermentation in the hindgut produces VFA and gases such as carbon dioxide. While VFA is absorbed gas appears as bubbles in feces giving feces the foamy appearance and loose consistency (Nordlund, 2004). In the present study it was observed that 85 per cent of SARA affected dairy cows had coarse fecal structure as against only 20 per cent in normal cows. This indicated that fecal structure in majority of SARA affected dairy cows tend to be coarse. This observation draws support from findings of Rossow (1984), Dirksen (1985), Nordlund et al. (1995), Oetzel (2000), Garry (2002), Kleen et al. (2003) and Tajik et al. (2008) who have reported that the feces of cattle affected SARA exhibits alteration in feces structure. The size of ingesta particles in SARA affected dairy cows tend to be larger than of normal dairy cows. The ingesta particle may be too large, being around 1-2 cm instead of less than 0.5cm and whole cereal grains may be also present in the feces (Kleen et al., 2003). The coarse fecal structure observed in SARA affected dairy cows can be attributed to reduced fiber digestibility as indicated by Nagaraja et al. (1978) and Ranjana et al. (2009) who did report that SARA reduces digestibility leading to alteration in fecal structure.

V. Conclusion

Rumenocentesis is a suitable method for collecting rumen fluid in dairy cows and pH of rumen fluid can be measured using portable pH meter. Diagnosis of SARA based on rumen fluid pH examination can be applied in the field condition. SARA resulted in alteration in rumen fluid with respect to Sedimentation activity time, Methylene blue reduction time, Iodophilic activity, Total protozoal count and Microbial composition. SARA might have reduced fecal pH, affects fecal consistency and fecal structure in majority of SARA affected dairy cows tended to be coarse.

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