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EVIDENCE OF TOXIC FACTORS IN THE RUMEN LIQUOR OF HEALTHY AND ACIDOTIC BUFFALO CALVES (BUBALUS BUBALIS)

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Abstract

Randhawa S. S., A. K. Ahuja, P. P. Gupta, S. S. Rathor: Evidence of Toxic Factors in the Rumen Liquor of Healthy and Acidotic Buffalo Calves (Bubalus bubalis). Acta vet. Brno, 56, 1987: 455-464.

Normal and acidotic rumen liquor samples from buffalo calves were subjected to various treatments and injected intraperitoneally to mice. The mortality rate was recorded up to 48 h of post injection. The toxic factors present in the acidotic rumen liquor were heat resistant and were found to be concentrated in the high centrifuged sediment. It was observed that the acidotic rumen liquor was about 1.63 times more toxic than the normal rumen liquor.

In the second experiment, the severity of toxicosis and intensity of histopathological alterations were quite apparent between 30 to 45 min. and death often occurred within 3 h of intraperitoneal injections. The toxic factors present in the acidotic rumen liquor might be endotoxic in nature.

Mice, i.p. administration, rumen liquor treatments, toxicosis.

Exclusive feeding of roughages to ruminants is associated with proliferation of Gram-negative bacteria whereas a sudden change to carbohydrate-rich diet results in predominance of Gram-positive flora (Hungate 1966; Slyter 1976). A number of toxic factors have been implicated in the pathogenesis of lactic acidosis in ruminants. The various compounds considered as possible toxic factors include lactic acid, biogenic amines, ethanol, bacterial toxins and unidentified toxic factors (Sanford 1963; Dunlop and Hammond 1965; Hungate 1966; Ahrens 1967; Slyter 1976; Nagaraja et al. 1979). These compounds may accumulate in the rumen due to microbial alterations associated with rumen dysfunction. A preliminary study was, therefore, carried out to assess the effect of normal and acidotic buffalo rumen liquor on mice mortality and to identify the nature of toxic factors that are lethal to mice on clinico-pathological basis.

Table 1 Effect of Treated Normal and Acidotic Rumen Liquor on Mice Mortality

Treatment of R.L.		Percent Mortality of Mice*	
		Normal R.L.	Acidotic R.L.
(i)	Seitz Filtered	0	0
(ii)	Boiled	20	100
(iii)	Strained (As such)	60	80
(iv)	Low Speed Centrifuged supernatant	60	100
(v)	High Speed Centrifuged supernatant	40	20
(vi)	High Speed Centrifuged sediment	60	100
(vii)	Control (Pyrogen Free D.W.)	0	0

* Results expressed as No. of Mice dead in 48 h/No injected X 100.
Total No. of Mice in each treatment-5

Materials and Methods

Acute lactic acidosis was induced in buffalo calves by oral feeding of molasses at the rate of 15 g/kg body weight. Rumen liquor samples were collected at 12 and 24 h post-feeding from healthy and acidotic buffalo calves with the help of a stomach tube. These samples were strained through four layers of muslin cloth, pooled and used for this study.

In the first experiment normal and acidotic rumen liquor was subjected to various treatments, viz. Seitz filtered, boiled (5 minutes); as such; low-speed centrifuged supernatant (2000 rpm for 5 minutes); high-speed centrifuged supernatant (18000 rpm for 20 minutes) and high-speed centrifuged sediment. The volume in each treatment was restored to the original level of the rumen liquor with pyrogen-free normal saline solution.

Seventy albino mice of both sexes (20-28 g) were procured locally. They were maintained on a commercial pelleted feed and had free access to water. On the basis of body mass, they were randomly divided into six duplicate groups (normal and acidotic) having five mice in each group. The rumen liquor samples subjected to various treatments as described above were intraperitoneally injected at the rate of 0.5 ml/mouse. The clinical symptoms and mortality rate were recorded up to 48 h.

In the second experiment, the lethality of strained rumen liquor, drawn from normal (roughage based) and lactic acidotic buffalo calves was tested with another set of 66 mice. Serial dilutions (0.125, 0.250, 0.500, 0.750 and 1.0 ml) of differently treated, normal and acidotic rumen liquor were made and the volume was raised to 1 ml with pyrogen-free normal saline. A group of six mice was subjected to each of the above treatments. In addition, a control group was also maintained in which the mice were given intraperitoneal injections of pyrogen-free normal saline. A close observation on clinical symptoms and mortality was recorded up to 48 h post injection. The LD_{16} and LD_{50} of normal and acidotic rumen liquor was calculated as per the method of Litchfield and Wilcoxon (1949).

In both experiments, representative tissues from each group were processed for histopathological examination as per conventional procedure.

Results

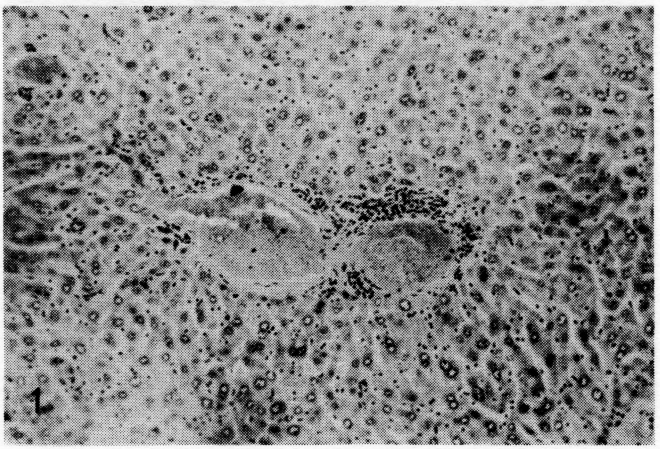
Intraperitoneal injections of strained rumen liquor from healthy or acidotic buffalo calves was lethal to mice within 48 h but the rumen liquor samples from acidotic calves were more toxic than those of roughage fed calves (Tables 1 and 2). The LD_{16} and LD_{50} values of normal rumen liquor were 0.13 and 0.31 ml whereas that of acidotic rumen liquor were 0.07 and 0.19 ml, respectively. The lethality results reflected that the acidotic rumen liquor was 1.63 times (LD_{50}) more toxic than the normal rumen liquor. No mortality was recorded in the control, Seitz-filtered groups with normal as well as acidotic rumen liquor. However, the mortality was 100 percent in mice injected with boiled acidotic rumen liquor, indicating that toxic factor was heat resistant. The effect of toxins present in high centrifuged sediment equalled that of the original strained rumen liquor. In the high centrifuged sediment from acidotic group, 100% mortality was recorded as compared to only 20% in high centrifuged supernatant group.

Table 2 Lethality to Mice of Normal and Acidotic Rumen Liquor from Buffalo calves

Dose of RL injected in Mice (ml)	Percent Mortality of Mice	
	Normal R.L.	Acidotic R.L.
0.125	16.6	33.3
0.250	50.0	66.6
0.500	66.6	83.3
0.750	83.3	100.0
1.000	100	100
Control	0	0

No. of mice in each treatment 6

Percent Mortality expressed as $\frac{\text{No. of Mice dead in 48 h}}{\text{No. of mice injected}} \times 100$



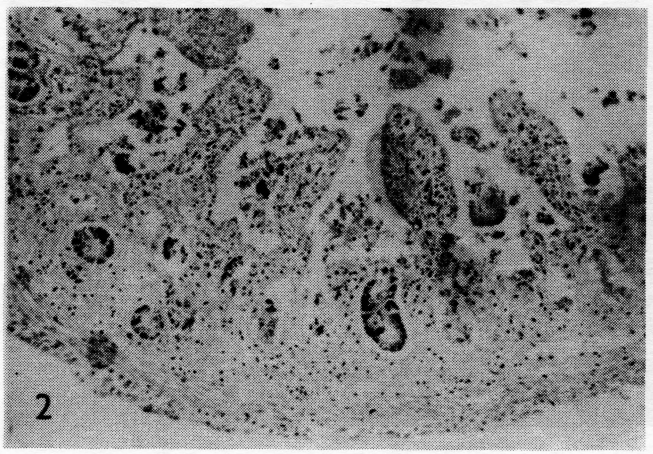
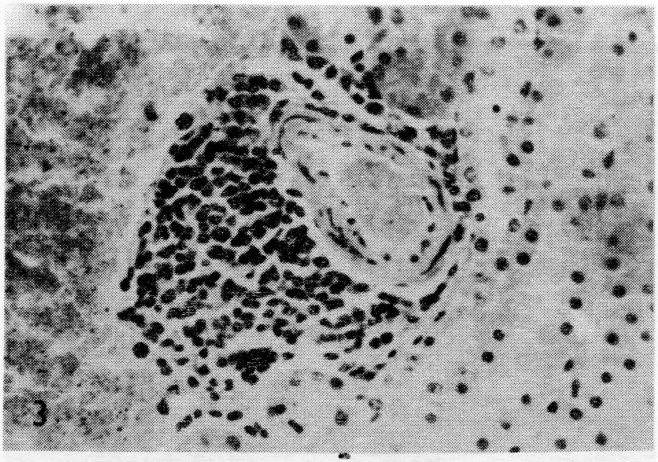


Fig. 1. Section of liver showing portal hepatitis H.E. X 70.

Fig. 2. Section of intestine showing necrotic enteritis of all the layers H.E. X 70.



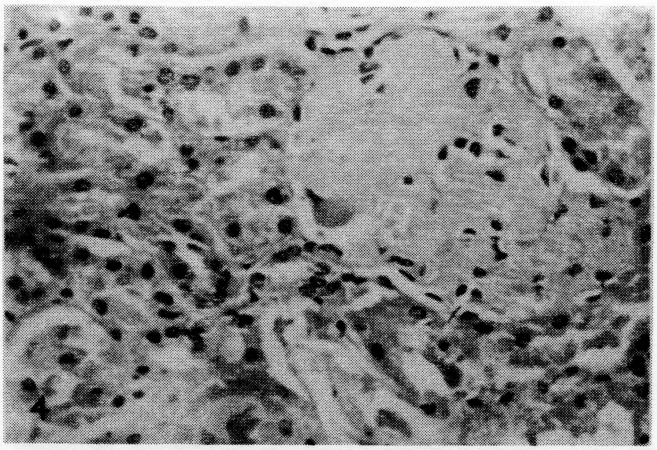


Fig. 3. Section of kidney showing perivascular infiltration of lymphocytes H.E. X 300.

Fig. 4. Section of kidney showing nephrotic changes in the tubular epithelial cells and necrosis of glomerulus H.E. X 300.

In the second experiment, 100% mortality was recorded with intraperitoneal injection of 1.0 ml normal rumen liquor as compared to 0.75 ml of acidotic rumen liquor. The onset and severity of signs of toxicosis and intensity of histopathological alterations depended upon the amount of rumen liquor injected and the type of treatment given. Usually the signs were observed between 30 to 45 minutes and death often occurred within 3 h of i.p. injection of low speed supernatant and high speed sediment component of the rumen liquor. The clinical signs of toxicosis were in the form of marked dullness and depression, reflected by the decreased activity and disinclination to eat or drink. This was followed by symptoms of ataxia associated with generalized muscular weakness and hyperventilation with dyspnoea. In the terminal stages, complete immobilization with unresponsiveness to external stimuli was observed. Some of the mice exhibited lateral recumbency and occasional convulsions just before death. However, the control mice given the normal saline solution did not show any ill effects.

No significant gross lesions were detected during necropsy except for congestion of the intestine observed in some of the mice. The histopathological examination revealed that intensity of the lesions was more marked in the high centrifuged sediment as well as low centrifuged supernatant rumen liquor injected groups as compared to the boiled and untreated rumen liquor injected groups. Histopathological alterations in the liver sections revealed fatty change, congestion and foci of coagulative necrosis with disorganisation of hepatic cords and non-purulent portal hepatitis (Fig. 1). The examination of lungs revealed severe congestion, pulmonary oedema, haemorrhagic and interstitial pneumonia with marked infiltration of lymphocytes and plasma cells around the blood vessels and the bronchioles, and in the lung parenchyma. Necrotic enteritis of all the layers of intestine (Fig. 2) was observed in mice that succumbed to the effect of toxicosis.

The sections of kidneys revealed nephrotic changes in tubular epithelial cells with focal non-purulent interstitial nephritis and perivascular lymphocytic cuffing (Fig. 3). Necrosis of glomeruli (Fig. 4) was also observed in some of the mice. No abnormal changes were detected on histopathological examination of heart and brain injected with untreated and boiled, normal as well as acidotic rumen liquor, whereas in other groups, areas of foci of coagulative necrosis of cardiac muscles (Fig. 5) and non-purulent pericarditis with infiltration of plasma cells and lymphocytes was observed. Lymphocytic meningo-encephalitis and acute necrotic gastritis (Fig. 6) were also observed.

Discussion

The results of the biochemical analysis revealed that the rumen liquor from normal calves was devoid of ethanol and contained only negligible quantities of histamine and lactic acid. But intraperitoneal injection of this liquor to mice also showed symptoms of toxicity. Similarly non-toxic to least toxic nature of the cell free high centrifuged supernatant fraction of rumen fluid and other metabolites such as volatile fatty acids, lactic acid and histamine are the possible factors responsible for mouse mortality. The non-toxicity of the Seitz filtered normal and acidotic rumen liquor and heat

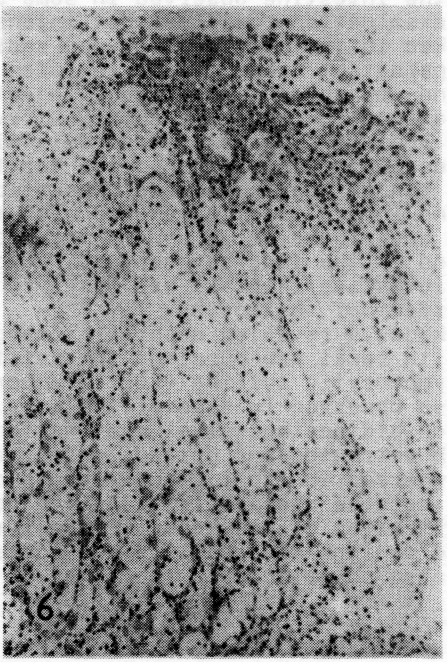


Fig. 5. Section of heart showing necrosis in myocardium H.E. X 70.

Fig. 6. Section of stomach showing necrotic gastritis H.E. X 70.

supernatant, reflected by high mortality revealed that toxic factors were concentrated in the bacterial fractions which possessed the properties of endotoxins. Such observations have also been made by Mullenax et al. (1966) and Nagaraja et al. (1979) with cattle rumen fluid.

It was also observed, on Gram's staining that normal rumen liquor contained a higher proportion of Gram-negative bacteria. Predominantly Gram-negative isolates were also found on aerobic cultural isolation of the normal rumen liquor. However, in the acidotic rumen liquor there was a shift in the proportion with predominance of Gram-positive bacteria, as confirmed by the aerobic cultural isolation studies. These observations also supported the results of lethality to mice with normal and acidotic rumen liquor indicating thereby that a qualitative increase in the concentration of toxic factors in the acidotic rumen fluid occurred probably due to disintegration of Gram-negative bacteria in the acidotic ruminal environment (Slyter 1976 and Nagaraja et al. 1979). The presence of toxic factor in the normal and acidotic rumen liquor as well as clinical symptoms and histopathological alterations observed in mice are comparable to the findings of Mc Manus et al. (1979), Nagaraja et al. (1978) and Nagaraja et al. (1979). These changes could be ascribed to the effect of ruminal bacteria and/or their toxic factors. However, the severity of toxic factors in the acidotic rumen liquor was not as marked as observed by these workers. This could be attributed to the fact that the degree of lactic acidosis reflected by the very low rumen liquor pH, was more intense in the present study. This might be due to the different sampling timings (12 to 24 h) in the present study as compared to 6 h by other workers. A marked increase in the rumen fluid accumulation due to increased intraruminal osmotic pressure in the present study might have resulted in the dilution of toxic factors in the acidotic animals which explains the variability in the degree of toxicity produced by acidotic rumen fluid.

It may be postulated that the toxic factors, though present in the intact rumen of healthy buffalo calves, are not exerting any undersirable effect but under conditions of carbohydrate engorgement more production and subsequent absorption of toxic factors from the inflamed and denuded rumen epithelium might lead to physiological disturbances, thus resulting in the shock-like syndrome which ultimately led to the loss of animals.

Průkaz toxických faktorů v bachorové tekutině zdravých a acidotických telat buvolů (Bubalus bubalis)

Vzorky bachorové tekutiny zdravých a acidotických telat buvolů byly ošetřeny různým způsobem a intraperitoneálně injikovány myším, u nichž byla v následujících 48 h sledována mortalita. Toxické faktory přítomné v acidotické bachorové tekutině byly tepelně resistentní vůči teplu a byly koncentrovány v odstředěném sedimentu. Acidotická bachorová tekutina byla asi 1,63 x toxičtější než tekutina ze zdravých telat.

V druhém pokusu měla toxikóza prudký průběh a patohistologické změny byly zjevné během 30 až 45 minut po aplikaci bachorové tekutiny. Pokusné myši hynuly během 3 h. Toxické faktory v acidotické bachorové tekutině mohou být svou povahou endotoxiny.

Определение токсических факторов в соках рубца здоровых и ацидотических буйволенков (*Bubalus bubalis*)

Образцы соков первого желудка здоровых и ацидотических буйволенков исследовали разными способами и интраперитонеально вводили мышам, у которых в последующие двое суток наблюдали смертноцть. Присуствующие в ацидотическом соке рубца токсические факторы отличались термической резистентностью к теплу и были сконцентрированы в центрифугированном осадке. Ацидотическая жидкость рубца по сравнению с соком здоровых буйволенков отличалась в 1,63 раза большей токсичностью.

В ходе второго эксперимента наблюдали более быстрое протекание токсикоза и патологические изменения проявились в течение 30 - 45 минут. Подопытные мыши погибали в течение 3 часов после применения сока первого желудка. Токсические факторы в ацидотическом желудочном соке можно по своему характеру считать эндотоксинами.

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