

STUDIES ON RUMINAL ATONY IN CATTLE AND ITS THERAPY

Thesis

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MASTER OF VETERINARY SCIENCE

in

**VETERINARY MEDICINE
(Minor Pharmacology)**

by

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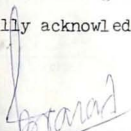
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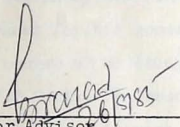
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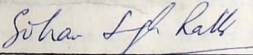
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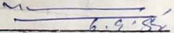
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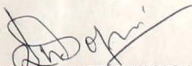
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(KANWAL KRISHEN SOPORI)

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1. INTRODUCTION

Cattle wealth is the backbone of Indian Agriculture. The economy of our country is mostly dependant on agriculture as well as animal production. In order to achieve self-sufficiency in milk production, greater emphasis is being laid on the improvement of genetic potential of the indigenous cattle through crossbreeding with exotic milch breeds. Consequently milk production has increased. To maintain the milk production, optimum supply of sufficient nutrients of good quality is essential. During extreme and adverse climatic conditions, the cattle are forced to thrive on poorest quality roughages.

Although exact statistical data is not available but there is no doubt that the diseases of digestive system cause great economic loss by way of low production and poor weight gain. The percentage of digestive disorders ranks the highest among non-infectious ailments in ruminants. Generally the common rumen dysfunctions are the outcome of sudden change in quality and/or quantity of feed. A sudden change in feed or fodder brings about a marked microbial and physico-chemical changes in the rumen leading to ruminal atony (Hoflund, 1967). The incidence of clinical cases of this condition have also been clinically recorded by Misra and Tripathy (1963), Prasad *et al.*, (1975) and Prasad (1977).

In rural areas, the cattle are exclusively maintained on wheat bhusa or paddy straw during the period of

non availability of green fodders and pasture grasses. Due to high lignification the digestibility of most of the nutrients is low in straws (Malik, 1984). Hence continuous feeding of straws not only cause the deficiency syndromes but also upset the digestive system leading to ruminal stasis. Cattle maintained exclusively on wheat straw develop in-appetence, decreased milk production and gradual loss of body condition. The correlation between rumen motility and rumen fluid characteristics often provides a measure for establishing the degree, duration and differential diagnosis of rumen dysfunction (Leek, 1983). Fundamentally the rumen motility is considered to be an index of digestive function in ruminants (Blood et al., 1983).

Efforts have been made to study some of the biochemical and therapeutic aspects on ruminal atony. There appears paucity of understanding the pathogenesis of ruminal atony due to exclusive feeding of wheat straw. A suitable therapy for such a condition has to be worked out to shorten the course of disease and to restore early normal function of the rumen.

Keeping in view the above factors, and in order to explore the various aspects of this clinical condition, the present project has been planned to study :

- (i) The effects of exclusive feeding of wheat straw in cattle.
- (ii) A detailed study of clinical symptomatology.

- (iii) Physical, microbial and biochemical changes in the rumen liquor.
- (iv) To monitor some biochemical changes in the blood.
- (v) Routine examination of urine.
- (vi) And to formulate a suitable line of treatment based on the findings on the untreated animals.

2:0 REVIEW OF LITERATURE

2:1 Ruminal atony in ruminants

At the beginning of current century, the rational reasons to cause various rumen dysfunctions were not ascertained. It was Fincher in the year 1940, who for the first time narrated various synonyms applied to diseases of digestive tract in bovines based on clinico-pathological observations like atony of forestomachs, acute indigestion, overloading of forestomachs, acute tympany and acute indigestion with tympany.

Misra and Tripathy (1963) could induce ruminal atony experimentally by exclusive feeding of paddy straw in cattle within a period of four weeks.

Hoflurd (1967) classified the rumen digestive disturbances in cattle on the basis of changes in the ruminal pH as

- (i) Acid indigestion (pH 4.0 - 5.5),
- (ii) Alkaline indigestion (pH 7.5 - 8.5) and
- (iii) Indigestion with normal pH 6.0 - 7.0.

Gibbons (1970) classified the primary rumen dysfunctions broadly into four categories like simple indigestion, indigestion with impaction, indigestion with tympany and indigestion with toxæmia in bovines.

In our country seasonal influence has been attributed to cause indigestion with impaction in cattle due to the change in climate to hot and dry with excessive feeding of

coarse roughages during summer (Joshi and Misra, 1974). The seasonal variation in incidence of indigestion in bovines has also been reported by Fincher and Fox (1963) and Pearson (1971).

Sethuraman and Rathor (1979) observed ruminal stasis in both cattle and buffaloes due to rise in pH of rumen.

2:2 Clinical symptomatology

Exclusive feeding of coarse, dry roughages to bovines, not only causes deficiency syndromes but also leads to ruminal stasis and upsets the digestive system. The clinical manifestations of such a condition have been described by many workers.

Misra and Tripathy (1963) recorded symptoms like dullness, depression, progressive emaciation and poor health, rough hair coat, constipation and anorexia in clinical cases of rumen dysfunction in cattle. The similar symptoms have also been described by workers like Joshi (1970); Sethuraman (1976); and Prasad (1979).

Blood et al. (1983) described the similar symptoms with recumbency and dyspnoea in terminal stages of the diseases.

2:3 Changes in rumen liquor

2:3:1 Physical changes

The physical characteristics of rumen fluid depend on the type and nature of feeds provided. Even in healthy

animals, maintained on normal diet, considerable variations in physical characteristics of rumen liquor has been reported (Dukes, 1955).

Misra and Tripathy (1963) recorded change in colour and reaction of rumen liquor depending on the type of feed, however the normal rumen liquor appeared viscous in consistency with aromatic smell.

Misra et al. (1972a) observed normal colour of rumen liquor as yellowish brown having aromatic odour and viscous in consistency. During indigestion and ruminal stasis, the colour changed to brown and sedimentation activity time (SAT) increased considerably.

Prasad and Rehib (1973) indicated SAT as a direct measure of disturbance in the rumen microbial activity. The abnormal rumen microbial digestion was diagnosed on the basis of cellulose digestion time (Rosenberger, 1979).

Leek (1983) described rumen fluid characteristics as an ideal index for establishing degree and differential diagnosis of rumen dysfunctions.

2:3:2 Rumen motility

Joshi and Ludri (1970) examined the rumen motility in clinical cases of indigestion in bovines and reported that the ruminal movements were either absent or infrequent in amplitude and frequency.

Sethuraman (1976) reported complete absence of reticulo-ruminal movements in clinical cases of alkaline indigestion in bovines maintained exclusively on coarse and dry roughages.

Prasad (1979) examined 74 clinical cases of primary anorexia syndrome in bovines and recorded ruminal movements one per two minutes (hypomotile) or absent in most of the cases.

McCarthy (1981) described the surface rippling of lower left flank of the cow as the mirror of ruminal motility.

Kay (1983) described rumen motility as the index for determining the digestive function in ruminants.

McSweeney (1983) observed decrease in forestomach motility after 4-6 hours in Lantanta poisoned sheep and motility of rumen remained depressed throughout the course of the disease.

Mohsin and Sud (1984) observed decrease in amplitude of rumen as well as reticular contractions under alkaline conditions in rumen in buffaloes.

2:3:3 pH of rumen fluid

Certain feeds and fodders are known to cause marked variation in the pH of rumen fluid in ruminants. Misra and Tripathy (1963) recorded increase in pH (upto 10) in adult cattle maintained on paddy straw for a period of about four weeks.

Joshi and Ludri (1970) recorded rumen pH in clinical cases of alkaline indigestion, which ranged from 8.0 to 9.5.

The normal pH of rumen liquor of cattle varied from 6.5 to 7.2 (Misra et al., 1972a ; Karunanidhi, 1983).

Prasad (1979) recorded ruminal pH of 6.7 to 8.2 in clinical cases of alkaline indigestion in cattle and buffaloes.

Choudhuri et al., (1981) recorded pH values of 8.0 to 8.9 in clinical cases of alkaline indigestion in bovines.

2:3:4 Microbial changes

Krogh (1963) reported absence of protozoa and variations in bacterial population in cases of rumen dysfunction in cattle.

Misra and Tripathy (1963) recorded poor motility and thin concentration of rumen infusoria in cases of indigestion in cattle. They further reported the absence of ciliate protozoa in case of rumen dysfunctions due to exclusive feeding of paddy straw in cattle.

Sethuraman (1976) observed decreased protozoal count as the ruminal pH crossed 8.0 in experimental alkalosis in bovines. The Gram positive bacteria also disappeared completely and Gram negative bacterial pattern dominated.

Choudhuri et al. (1981) found only inactive and dead protozoa in cows suffering from alkalosis and rumen dysfunction.

Sethuraman et al. (1980) cultured rumen fluid of cattle suffering from alkalosis and found predominance of *Escherichia coli*, *Proteus*, *Pseudomonas* and *Actinobacillus*.

Blood et al. (1983) described presence of few dead protozoa or complete absence of protozoa in cases of indigestion with impaction.

2:3:5 Biochemical changes in rumen liquor

2:3:5:1 Total volatile fatty acids (TVFA)

Volatile fatty acids are the byproducts of microbial fermentation of ingesta in rumen, which serve as the main source of energy for the host animal.

Annison and Lewis (1959) reported that the level of rumen volatile fatty acids was dependant on the type of ration fed to the animals.

Joshi and Misra (1975) found a negative correlation between TVFA and ruminal pH in clinical cases of rumen dysfunction in cattle.

Sethuraman (1976) recorded a concentration of 5.0 m Eq/L of TVFA at 96 hour following experimental induction of ruminal alkalosis in bovines.

Prasad (1977) reported negative correlation between TVFA and ruminal pH in spontaneous rumen dysfunction in

cattle and buffaloes.

A considerable decrease in TVFA concentration in case of experimentally induced ruminal alkalosis has been reported by Dave (1980) in buffalo calves.

The TVFA concentration of rumen liquor in healthy crossbred cow calves has been established as 68.46 ± 4.13 mEq/L by Karunanidhi (1982).

2:3:5:2 Ammonia nitrogen in rumen fluid

In ruminants dietary proteins and NPN substances are acted upon and utilised by rumen microbes and appreciable quantity of ammonia is produced in the rumen (Hungate, 1966); (Tillman and Sidhu, 1969).

Joshi and Ludri (1970) recorded ammonia nitrogen concentration upto 54.05 mg % in clinical cases of alkaline indigestion in bovines.

Chalmers et al. (1971) suggested that ammonia might reach the peripheral blood via lymphatics after its absorption from rumen into peritoneal cavity.

Lewis and Buttery (1972) suggested that simple diffusion as sufficient explanation for the transport of large quantities of ammonia across the rumen wall, however existence of an active transport system could not be ruled out.

Joshi and Misra (1975) reported increase in ammonia nitrogen level in clinical cases of indigestion with higher

pH. The similar findings have also been conveyed by Prasad (1977) and Dave (1980).

Prasad (1977) reported a negative correlation between total volatile fatty acids (TVFA) and ammonia nitrogen ($\text{NH}_3\text{-N}$) and a positive correlation between pH and $\text{NH}_3\text{-N}$ of the rumen in clinical as well as experimental alkaline indigestion in cattle and buffaloes.

Bueno et al. (1977) observed inhibition of reticulo-ruminal contractions in four fistulated sheep by intra-ruminal administration of ammonia solution.

2:3:5:3 Electrolytes (Sodium and Potassium)

Sethuraman (1976) observed fluctuating values of both sodium and potassium in rumen liquor of bovines with experimentally induced alkalosis. In clinical cases the sodium and potassium level ranged from 280 to 310 and 48 to 58 mg % respectively.

Choudhuri et al. (1981) recorded levels of electrolytes in rumen fluid of normal buffalo calves as sodium 155 mEq/L and potassium 35.60 mEq/L.

Karunanidhi (1982) recorded mean values of sodium and potassium in rumen liquor as 151.35 ± 1.08 and 35.80 ± 0.79 respectively in healthy crossbred calves.

2:3:5:4 Calcium, Magnesium and inorganic phosphorus

Wilson (1963) reported that high rumen ammonia did not influence the availability of dietary magnesium.

Contrary to this high rumen ammonia concentration caused significant effect on the absorption of calcium, magnesium and phosphorus from the digestive tract (Fontenot and Webb, 1971) and (Moore et al., 1972).

Salma (1977) reported decrease in rumen magnesium level from 11.7 to 2.6 mg % with a concomitant decrease in calcium level in sheep when given magnesium deficient diets.

Martens (1980) observed existence of negative linear relationship between magnesium absorption and $\text{NH}_3\text{-N}$ concentration, and a positive linear relationship between magnesium absorption and TVFA concentration in the rumen of sheep.

Dave (1980) recorded no significant change in calcium, magnesium and inorganic phosphorus level of rumen liquor in urea induced alkalosis in buffalo calves.

Dietary phosphorus deficiency reduced the efficiency of intestinal calcium absorption in sheep (Abdel-Hafeez et al., 1982).

2:3:5:5 Thiamine in rumen liquor

Bachdel and Honeywell (1927) and Bachdel (1928) reported the possibility of thiamine synthesis by rumen flora.

Kon and Porter (1954) stated that most of the ruminal thiamine was absorbed from rumen.

Philipson and Reid (1957) stated that most of the ruminal thiamine was extracellular the concentration of which was directly proportional to the thiamine content of food ingested.

Hungate (1966) was of opinion that synthesis of thiamine by rumen microbes was not sufficient to meet the total requirement of the ruminants.

Hoflund (1967) suggested a possible inhibition of thiamine synthesis during digestive disorders in ruminants.

Edwin and Jackman (1970) attributed enzyme thiaminase type I, produced by *Clostridium sporogenes* and certain other bacteria, to cause hypothiaminosis in ruminants.

Morgan and Lawson (1974) also noticed thiaminase, which destroys thiamine in rumen liquor.

Naga et al. (1975) stated that rations deficient in thiamine content lead to anorexia and symptoms of cerebro-cortical necrosis in sheep.

Quaghebeur et al. (1975) suggested that thiamine destroying activity in the rumen fluid was due to thermostable compounds dissolved in the rumen fluid, the activity of which depended greatly on the pH of the medium and thiamine concentration. However the exact nature of compounds could not be established.

Herrick (1975) reported that biosynthesis of thiamine in the rumen was influenced by type and composition

of feeds and change of pasture, as they differentially influenced the growth of rumen microbes. He further suggested that thiamine anti-metabolites present in fungi and bacteria grown on spoiled feeds could block the synthesis or absorption of thiamine in rumen.

Gupta et al. (1976) recorded significant decrease in rumen thiamine concentration in cattle from 7.04 ± 1.04 to 1.99 ± 0.33 mcg per cent in rumen dysfunctions. They attributed the dearranged synthesis and destruction of thiamine in rumen for such decrease.

Karunanidhi (1982) also reported decrease in ruminal thiamine level in case of acidosis in calves.

Blood et al. (1983) described decreased level of thiamine in case of indigestion in cattle leading to higher level of blood pyruvic acid.

2.4 Changes in blood/serum

2:4:1 Ammonia nitrogen in serum

Lewis (1960) reported that when blood ammonia nitrogen level exceeded 0.8 mg %, the toxic symptoms were observed in sheep.

Hogan (1961) and Bloomfield et al. (1963) observed that the absorption rate of ammonia into blood was a pH dependant phenomenon and it occurred at rumen pH above 7.0.

Webb et al. (1972) noticed a significant positive correlation between rumen and peripheral blood ammonia levels.

Dave (1980) reported toxic signs of hyperammonaemia in experimental rumen alkalosis in buffalo calves when serum ammonia nitrogen concentration reached 1.93 ± 0.399 mg %.

2:4:2 Electrolytes (Sodium, potassium)

Huber (1971) reported isotonic loss of sodium, potassium and chloride concentration in serum of experimentally induced indigestion in sheep.

Cakala et al. (1974) observed decreased concentration of both sodium and potassium in serum of six cows with ruminal dysfunction.

Sethuraman (1976) reported non-significant decrease in the concentration of sodium and potassium in experimental ruminal alkalosis in bovines.

Davidovich et al. (1977) recorded non-significant change in the concentration of serum sodium and potassium in cattle suffering from rumen dysfunction.

Dave (1980) also observed non-significant change in serum sodium and potassium level in experimentally induced alkalosis in buffalo calves.

Kuiper (1980) suggested increased urinary excretion of sodium leading to dehydration in case of experimentally induced metabolic alkalosis in the cows.

Cakala et al. (1980) observed increase in serum sodium and decrease in potassium, calcium, magnesium and

inorganic phosphorus level in experimentally induced alkalosis in cattle.

2:4:3 Calcium, Magnesium and inorganic phosphorus

Moore et al. (1972) found non-significant alterations in serum calcium, magnesium and inorganic phosphorus concentration.

Prasad et al. (1972) estimated serum calcium and phosphorus in clinical cases of rumen dysfunction in bovines. They found serum calcium concentration varying from 9.1 to 10.47 mg % and inorganic phosphorus 3.9 to 4.3 mg %.

Joshi and Misra (1973) analysed the serum samples collected from clinical cases of indigestion with atony in cattle and recorded non-significant correlation coefficient between rumen pH and serum calcium and phosphorus concentration.

Horn and Smith (1976) reported poor absorption of magnesium in ruminants when pH of rumen was high.

Sethruman and Rathor (1979) mentioned decrease in serum calcium and phosphorus in experimental alkaline indigestion in cattle when compared with zero hour values. They attributed poor absorption from intestine for the decrease.

Regier (1980) noted decreased absorption of magnesium from rumen in presence of ammonia, while the presence of volatile fatty acids increased the absorption.

Martens (1980) observed negative linear relationship between magnesium absorption and ammonia nitrogen concentration and a positive linear relationship between magnesium absorption and VFA in the rumen of sheep.

Dave (1980) did not observe any alteration in serum calcium, magnesium and inorganic phosphorus level in urea induced ruminal alkalosis in buffalo calves.

Huber et al. (1981) recorded cessation of rumen motility in experimentally induced hypocalcaemia before the classical signs of hypocalcaemia could be noted. Hence he opined that the ruminal dysfunctions occur considerably before the onset of clinical signs of hypocalcaemia.

Daniel (1983) stated that the rate and the amplitude of rumen contractions was greatly reduced by decrease in plasma calcium level. He further reported a significant linear relationship between the rate and amplitude of rumen contractions over a plasma calcium range.

2:4:5 Thiamine

Gupta et al. (1976) reported a significant decrease in the concentration of thiamine in blood in clinical cases of indigestion in cattle. The value of thiamine decreased from 3.35 ± 0.03 to 2.43 ± 0.35 mcg%.

A significant decrease in thiamine concentration of blood was recorded by Sinha (1981) in buffalo calves due to acidosis.

Karunanidhi (1982) also reported significant decrease in thiamine concentration of blood in experimentally induced acidosis in calves.

2:4:6 Urinalysis

Singer and McCarthy (1971) observed haematuria and albuminuria in sheep suffering from acute ammonia toxicity.

Dave (1980) reported traces of albumin as the lone pathological constituent in urine samples obtained from experimental and clinical cases of alkalosis in buffaloes.

2:5 Therapy of ruminal atony

Clark et al. (1951) administered 5% acetic acid into the rumen directly in sheep suffering from urea toxicity and alkalosis. They found it to be beneficial even when the symptoms were advanced.

Hoflund (1967) suggested oral administration of 6% lactic acid, streptomycin preparation 6-10 g, molasses and fresh rumen cud for 3-5 days daily as a line of treatment for alkalosis in cattle.

Kadvekar et al. (1971) treated clinical cases of anorexia and rumen dysfunction with 1 ml of vitamin-B-complex and 1 ml of liver extract per 50 kg body weight intramuscularly. All the animals recovered after 3rd injection.

Misra and Singh (1974) treated clinical cases of alkaline indigestion in cattle by drenching 200 ml of 5%

lactic acid along with Himalayan Batisa 40 g orally daily for six days and 2-3 ml of Livogen (Glaxo) intramuscularly on alternate days.

Singh and Thakur (1974) treated a case of ruminal stasis due to urea poisoning in heifer with oral administration of 750 ml vinegar and 200 ml of calcium borogluconate intravenously.

Joshi (1976) treated successfully clinical cases of ruminal atony in bovines with a mixture of strychnine sulphate, cobalt sulfate, manganese chloride, sodium bicarbonate and sodium phosphate given orally twice daily for three days. He, further commented that the preparation stimulated microbial activity in rumen.

Prasad et al. (1976) administered Anorexon (Pfizer) one tablet twice daily orally in cattle and buffaloes and recorded good result in clinical cases of primary rumen dysfunction.

Bartley et al. (1976) evacuated the rumen contents before the onset of symptoms in experimentally induced alkalosis in cattle and then drenched acetic acid 5%. The rumen fermentation started within 48 hours of treatment as judged from ruminal pH, reticulo-ruminal motility and odour of rumen contents.

Sethuraman (1976) treated successfully the cases of experimentally induced alkalosis in bovines with acetic

acid 10% @ 10 ml per kg. body weight, streptomycin sulphate 1 g per 50 g body weight and a mixture of magnesium sulphate and sodium sulphate orally along with Ringer lactate intravenously and Promethazine hydrochloride intramuscularly on the first day. Subsequently rumen cud transplantation and liver extract intramuscularly were also used for toning up of liver and restoration of protozoal population.

Davidovich et al. (1977) advocated emptying of rumen contents in cases of indigestion with impaction.

Mohsin and Sud (1978) studied the effect of different drugs on reticulo-ruminal motility in the buffaloes and recommended administration of Carbachol at the rate of 2 mg per 100 kg body weight, Arecoline hydrobromide @ 4-8 mg per 100 kg body weight and Eserine salicylate @ 20 mg per 100 kg body weight. They recorded that the use of these drugs, in these dosage regimens, increased amplitude as well as frequency of reticulo-ruminal contractions.

Choudhuri et al. (1981) treated successfully clinical cases of ruminal alkalosis in cows with 500 ml of lactic acid (2%) and 2 litres of fresh rumen liquor orally along with other supportive treatment for 5-10 days.

Dave (1980) treated buffalo calves suffering from alkalosis by siphoning out 50% of ruminal contents through

rumen fistulae and oral supplementation of 200-400 ml of 5% glacial acetic acid (BDH) along with intramuscular infections of Livogen (Glaxo) @ 1 ml per 50 kg body weight once daily and two litres of fresh rumen liquor intraruminally for three days. However in clinical cases of ruminal atony only acetic acid 5% and Livogen were used along with fresh drinking water and chopped green fodder.

The principles underlined for the treatment of digestive disorders of ruminants like correction of pH of rumen to normal range, establishment of ruminal tone, destruction of pathogenic microbes of rumen, correction of dehydration and toxæmia, reestablishment of normal activity of rumen microbes and toning up of liver, were recommended by Blood et al. (1983).

3:0 MATERIAL AND METHODS

3:1 Experimental animals

Fifteen healthy male crossbred calves of 1-1½ years age were procured for the present study. The animals were maintained on chopped green fodder and reared under uniform managemental conditions for a period of one month. They were examined clinically and blood, faecal and urine samples were screened to rule out the presence of any disease. All the calves were fitted with sterilised polythene rumen-fistulae of 60 mm diameter on left paralumber fossa following the technique adopted by Roychoudhury (1981). Necessary post operative care was taken with local antiseptic dressing. A course of streptopencillin intramuscularly was given for five days to enhance the wound healing. In all the calves, the fistulation was successful. The animals were divided into three groups (Group I, II and III) comprising of five calves in each group. The calves of group I served as healthy control while in the five calves belonging to group II, ruminal atony was induced experimentally and different parameters were studied. In the calves of group III, ruminal atony was induced as in group II and treatment was undertaken based on the findings observed in the animals of group II.

3:2 Sampling material

Collection of rumen fluid, blood and urine samples was done from animals of first group at 48 hours interval

~~interval~~ for a period of nine days to establish the normal values.

About 30 ml of rumen fluid was collected from each animal and strained through double fold muslin cloth. Estimation of pH, sedimentation activity time (SAT), microbial analysis, ammonia nitrogen ($\text{NH}_3\text{-N}$), and total volatile fatty acids (TVFA) were carried out immediately after collection of rumen liquor and the remaining samples were stored in deep freeze for further analysis.

About 15 ml of blood was drawn from jugular vein aseptically in a plain vial and serum was separated, centrifuged and kept for analysis.

About 30-50 ml of urine samples were collected from each animal as and when available and stored in glass bottles for routine analysis.

3:3 Analysis of samples

3:3:1 Rumen liquor

3:3:1:1 Physical changes

Colour, consistency and odour of the rumen liquor were recorded as per Rosenberger (1979). Sedimentation activity time (SAT) was noted as per the method of Nicholas and Penn (1958).

3:3:1:2 Microbial changes

The protozoan motility was observed under coverslip preparation of strained rumen liquor (SRL) and was graded

as vigorous (+++), moderate (++), sluggish (+) and nil (0) as per the method of Misra and Singh (1974). The concentration of protozoa was recorded as per method of Misra et al. (1972b) and was graded as under

- (+++) when 30 or more protozoa were seen per low power field (LPF),
 (++) 10-30 protozoa per LPF and
 (+) 1-10 protozoa per LPF.

Staining of rumen protozoa

Rumen protozoa were stained with 0.5% methyl green in 5% acetic acid (Hurgate 1966). The diluting fluid for staining rumen protozoa was prepared as under :

50% Formalin	=	0.8 ml
0.5% Methyl green	=	0.2 ml
30% Glycerine	=	3.0 ml
S.R.L.	=	1.0 ml

Total and Differential protozoal count

The method described by Moir (1951) was adopted for determining the total and differential protozoal counts. For this purpose standardisation of microscopic field was done by using micrometer and the factor was derived. The rumen protozoa were counted under low power and identified under high power for differential counts.

Bacterial pattern

As per Hungate et al. (1952) the rumen content was diluted 10 times with distilled water and 0.01 ml was spread over a clean glass slide. The smear was dried, fixed over a flame and Gram's method of staining was carried out to differentiate the rumen bacteria (Coles, 1979).

3:3:1:3 Biochemical changes in rumen liquor and blood.

Rumen pH

Immediately after collection the pH of rumen liquor was determined using an Elico-pH-meter.*

Total volatile fatty acids (TVFA)

The concentration of TVFA in the rumen liquor was estimated by method of Barnett and Reid (1957) using Markham's apparatus for steam distillation. The distillate collected in conical flask was titrated against 0.01N sodium hydroxide solution using phenol-phthalein as indicator.

Ammonia nitrogen (NH₃-N)

Ammonia nitrogen in rumen liquor and serum was estimated by micro-diffusion method of Conway (1957).

Sodium and potassium

The sodium and potassium content of serum and rumen

* pH meter Model L1-10 Elico Private Ltd., Hyderabad.

liquor was determined by Flame-photometric technique,** as described by Oser (1965). The diluted unknown samples were introduced in the form of continuous fine spray into non-luminous gas flame. The light wave length emitted characteristic of sodium and potassium was isolated by using the proper filter.

Thiamine

Thiamine concentration in rumen liquor and blood was estimated as per the method described by Myint and Houser (1965). After acid and enzymic hydrolysis the samples were treated with oxidising agent i.e. potassium ferricyanide and oxidised to thiochrome. The samples were processed as under :

5 ml of samples (SRL/serum) were taken in 40 ml capped test tubes. To this 3 ml of oxidising agent (4.0 ml potassium ferricyanide 1% + 96 ml 3.5 N sodium hydroxide) was added. Immediately (within thirty seconds) 20 ml of isobutyl alcohol was added and the contents were mixed vigorously for about 90 seconds. Two ml of dehydrated alcohol was added and tubes were swirled for few seconds. The fluid was allowed to settle. A Blank was also processed using distilled water and instead of oxidising agent 3 ml of 3.5 N sodium hydroxide was added and processed as above. The clear supernatant fluid was used for measuring

** Flame-photometer-Syntronics, Ahmedabad, India.

the fluorescence in Turner Fluorometer* set at excitation 350 m μ and emission 450 m μ wave lengths. The thiamine concentration was determined from the plotted standard curve.

Calcium

Rumen fluid and serum calcium concentration was estimated colorimetrically as per the method of Webster (1962).

Magnesium

Rumen fluid and serum magnesium content was estimated by Titan-Yellow method as mentioned by Oser (1965).

Inorganic phosphorus

Inorganic phosphorus in rumen liquor and serum was determined by Fiske and Subbarow method as described by Oser (1965).

3:3:1:4 Urine examination

Urine samples were analysed qualitatively for albumin, (Heller's test), glucose (Benedict's test), ketone bodies (Rothera's test) and bile as per Sastry (1983).

3:4 Experimental induction of ruminal atony in animals of group II

The animals belonging to this group were weighed before the experiment. In addition to the ad lib provision,

* Turner spectrofluorometer, Model 430, U.S.A.

intraruminal administration of wheat straw at the rate of 2 kg. per 50 kg body weight to each animal daily was done till there was complete stasis of rumen and reticulo-ruminal contractions in amplitude as well as frequency. Rumen fluid, blood and urine samples were collected on every alternate day, throughout the experiment. However, the clinical symptoms were recorded daily during entire course of disease. Analysis of rumen fluid, blood/serum and urine samples was carried out as described under 3:3.

3:5 Induction of ruminal atony and therapy in animals of group III

All the five animals were weighed before the experiment and atony of rumen was created as in animals of group II by intra-ruminal administration of wheat straw. The following line of treatment was adopted soon after the development of ruminal atony :

- 1) Intraruminal administration of 200-250 ml of 3% (v/v) glacial acetic acid for lowering the rumen pH.
- 2) Intravenous administration of Thiactal (WOCKHARDT) at the dose rate of 150-200 ml daily for three days.
- 3) Powder Digestovet (VETS PHARMA PVT.LTD.) and Bovirum bolus (Sarabhai Chemicals) at the dose rate of 40 g and one bolus respectively daily twice for three days.
- 4) Normal saline (LUNA PHARMA INDIA) 540-800 ml I/V to each animal for two days.

- 5) Provision of sufficient fresh drinking water and chopped green fodder daily.

Rumen fluid, blood and urine samples were collected as 3:2 before medication and then at 24 hours interval till complete recovery of animals. Analysis of samples was carried out as described under 3:3.

Statistical analysis

The data of group I i.e. healthy control crossbred calves were processed for mean values \pm S.E. Statistical analysis of data on group II and group III was carried out for stagewise variations using student's 't' test.

4:0 RESULTS

4:1 Observations on healthy control crossbred calves (Group I)

All the five calves of this group were examined clinically. Evaluation of rumen liquor in respect of physical characteristics, microbial activity and biochemical status was done. Biochemical analysis of blood and routine examination of urine were performed.

4:1:1 Clinical observations

Observations on different clinical parameters are presented in Table 1. The general condition, appearance, appetite, urination and defecation were found to be normal. The calves were quite active and alert clinically and no abnormality could be detected. The overall average rumen motility and body temperature were 3.08 ± 0.048 /two minutes and $39.14 \pm 0.022^{\circ}\text{C}$ respectively. The conjunctival mucous membrane appeared normal and roseate in colour in all the animals.

4:1:2 Observations on rumen liquor

4:1:2:1 Physical characteristics

The observations on physical characteristics of rumen liquor in healthy control crossbred calves are noted in table 2. The rumen liquor was viscous in consistency having aromatic odour and greenish brown colour. The mean sedimentation activity time (SAT) of rumen liquor was 14.36 ± 0.643 minutes.

Table 1. Clinical observations on healthy control crossbred calves (Group I)

Parameters	I ay(s) Mean \pm S.E.					Overall average
	1st	3rd	5th	7th	9th	
General condition	Normal and active	Normal and active	Normal and active	Normal and active	Normal and active	-
Abdomen	- No distension -					
Rumen motility/ 2 minutes	3.00 ± 0.00	3.00 ± 0.00	3.2 ± 0.20	3.00 ± 0.00	3.2 ± 0.00	3.08 ± 0.048
Temperature ($^{\circ}$ C)	39.11 ± 0.021	39.22 ± 0.011	39.11 ± 0.015	39.16 ± 0.011	39.11 ± 0.011	39.14 ± 0.022
Mucous membrane	Roseate	Roseate	Roseate	Roseate	Roseate	
No. of animals	5	5	5	5	5	

Table 2. Physical examination of rumen liquor of healthy control crossbred calves (Group I).

Parameters	Day(s) Mean \pm S.E.					Overall average
	1st	3rd	5th	7th	9th	
Colour	Greenish brown	Greenish brown	Greenish brown	Greenish brown	Greenish brown	
Consistency	Viscous	Viscous	Viscous	Viscous	Viscous	
Odour	Aromatic	Aromatic	Aromatic	Aromatic	Aromatic	
Motility	+++	+++	+++	++	+++	
Concentration	+++	+++	+++	+++	+++	
S.A.T. (minutes)	15.00 ± 0.961	16.4 ± 1.208	13.00 ± 0.836	13.00 ± 0.422	14.4 ± 0.509	14.36 ± 0.643
pH	6.90 ± 0.029	7.00 ± 0.061	6.79 ± 0.071	6.85 ± 0.025	6.90 ± 0.058	6.89 ± 0.035
No. of animals	5	5	5	5	5	5

Motility

+++ Vigorous
 ++ Moderate
 + Sluggish
 0 Nil

Concentration

+++ More than 30 protozoa per LPF
 ++ 10-30 protozoa per LPF
 + Less than 10 protozoa per LPF
 0 Nil

Table 3. Protozoal examination of rumen liquor of healthy control crossbred calves (Group I)

Parameters	Day(s) Mean \pm S.E.					Overall average
	1st	3rd	5th	7th	9th	
Total protozoal count ($\times 10^5$)	2.51 ± 0.112	2.92 ± 0.130	2.29 ± 0.212	2.79 ± 0.287	2.65 ± 0.431	2.63 ± 0.109
Differential count (%) (Mean \pm S.E.)						
Isotricha	10.2 ± 0.221	9.8 ± 0.281	11.6 ± 0.211	11.0 ± 0.254	11.2 ± 0.262	10.76 ± 0.331
Dasytricha	3.4 ± 0.291	4.4 ± 0.351	3.4 ± 0.355	3.2 ± 0.219	5.2 ± 0.365	3.92 ± 0.383
Ertodinium	63.8 ± 0.935	62.2 ± 0.857	65.2 ± 1.005	60.6 ± 1.015	60.4 ± 1.023	62.44 ± 0.924
Deplodinium	15.2 ± 0.312	15.4 ± 0.357	14.4 ± 0.295	17.0 ± 0.410	16.2 ± 0.381	15.64 ± 0.445
Epidinium	3.4 ± 0.281	4.0 ± 0.252	3.2 ± 0.265	5.2 ± 0.308	3.2 ± 0.211	3.8 ± 0.412
Unidentified and others	4.0 ± 0.447	4.2 ± 0.452	4.2 ± 0.452	3.0 ± 0.314	3.8 ± 0.412	3.84 ± 0.223
No. of animals	5	5	5	5	5	5

The motility of protozoa was observed to be vigorous (++++) and their concentration was directly proportional to their motility.

4:1:2:2 Microbial pattern

Table 3 depicts the microbial pattern of rumen liquor of healthy crossbred calves.

The mean total protozoal count was $2.63 \pm 0.109 \times 10^5$ per ml of strained rumen liquor while the differential pattern of rumen protozoa revealed. Isotricha $10.76 \pm 0.331\%$; Dasytricha $3.92 \pm 0.383\%$; Entodinium $62.44 \pm 0.924\%$; Diplodinium $15.64 \pm 0.445\%$; Epidinium $3.8 \pm 0.412\%$ and unidentified $3.84 \pm 0.223\%$.

Gram stained rumen fluid smears revealed presence of Gram negative bacteria predominantly and the types of bacteria observed were rods, cocci and coccobacilli. Some Gram positive rods and cocci were also noticed in the stained smears.

4:1:2:3 Biochemical analysis of rumen liquor

Results of biochemical analysis of healthy control crossbred calves are presented in Table 4.

The overall average of rumen liquor pH was 6.89 ± 0.035 . Overall concentration of ammonia nitrogen ($\text{NH}_3\text{-N}$) and total volatile fatty acids (TVFA) were 12.81 ± 0.323 mg % and 62.25 ± 1.409 mEq/L respectively. The mean values of sodium and potassium were 150.72 ± 1.361 and $35.09 \pm$

Table 4. Biochemical analysis of rumen liquor of healthy control crossbred calves (Group I).

Parameters	Day(s) Mean \pm S.E.					Overall Average
	1st	3rd	5th	7th	9th	
Ammonia nitrogen (mg%)	11.95 ± 0.461	13.75 ± 0.229	12.90 ± 1.092	13.20 ± 0.421	12.25 ± 0.415	12.81 ± 0.323
Total volatile fatty acids (mEq/L)	62.00 ± 1.125	60.80 ± 1.145	67.50 ± 1.951	61.82 ± 1.430	59.12 ± 1.018	62.25 ± 1.409
Sodium (mEq/L)	150.25 ± 3.281	146.00 ± 2.952	151.25 ± 3.402	151.75 ± 2.609	154.35 ± 3.128	150.72 ± 1.361
Potassium (mEq/L)	35.20 ± 0.809	30.72 ± 0.712	38.75 ± 0.419	33.85 ± 0.512	36.92 ± 0.991	35.09 ± 1.367
Calcium (mg%)	15.25 ± 0.685	15.70 ± 0.391	14.20 ± 0.521	14.70 ± 0.417	12.85 ± 0.359	14.54 ± 0.492
Magnesium (mg%)	6.92 ± 0.195	5.81 ± 0.270	5.46 ± 0.275	5.18 ± 0.715	6.10 ± 0.528	5.89 ± 0.300
Phosphorus (mg%)	9.10 ± 0.207	8.25 ± 0.202	8.75 ± 0.275	9.15 ± 0.228	8.10 ± 0.291	8.67 ± 0.215
Thiamine (mcg%)	6.25 ± 0.132	6.10 ± 0.119	5.65 ± 0.195	5.81 ± 0.192	6.06 ± 0.178	5.97 ± 0.108
No. of animals	5	5	5	5	5	5

Table 5. Biochemical analysis of blood/serum of healthy control crossbred calves (Group I).

Parameters	Day(s) Mean \pm S.E.					Overall average
	1st	3rd	5th	7th	9th	
NH ₃ -N(mg%)	0.42 \pm 0.012	0.41 \pm 0.016	0.40 \pm 0.018	0.44 \pm 0.119	0.41 \pm 0.113	0.42 \pm 0.001
Sodium (mEq/L)	130.25 \pm 2.921	125.75 \pm 3.106	131.00 \pm 2.505	142.22 \pm 3.411	130.75 \pm 3.269	131.89 \pm 2.789
Potassium (mEq/L)	3.91 \pm 0.171	3.75 \pm 0.112	4.30 \pm 0.281	4.53 \pm 0.213	4.10 \pm 0.115	4.12 \pm 0.139
Calcium (mg%)	9.51 \pm 0.224	10.25 \pm 0.361	9.86 \pm 0.541	10.75 \pm 0.252	10.81 \pm 0.275	10.24 \pm 0.251
Magnesium (mg%)	1.91 \pm 0.081	2.10 \pm 0.101	2.16 \pm 0.108	1.85 \pm 0.096	2.00 \pm 0.058	2.00 \pm 0.057
Phosphorus (mg%)	4.06 \pm 0.059	3.82 \pm 0.062	3.70 \pm 0.071	4.00 \pm 0.052	3.92 \pm 0.092	3.90 \pm 0.064
Thiamine (mcg%)	3.40 \pm 0.172	3.80 \pm 0.109	3.68 \pm 0.171	3.95 \pm 0.193	3.50 \pm 0.312	3.66 \pm 0.992
No. of animals	5	5	5	5	5	5

1.367 mEq/L respectively. The levels of calcium, magnesium and inorganic phosphorus were 14.54 ± 0.492 ; 5.89 ± 0.300 and 8.67 ± 0.215 mg per cent respectively while the average thiamine concentration was 5.97 ± 0.108 mcg per cent in rumen fluid.

4:1:3 Changes in serum/blood

Results of serum/blood biochemical analysis of healthy control crossbred calves are presented in Table 5. The respective values of calcium, magnesium and inorganic phosphorus were 10.24 ± 0.251 ; 2.00 ± 0.057 and 3.90 ± 0.064 mg per cent. Blood thiamine level was 3.66 ± 0.992 mcg per cent. Serum sodium and potassium levels were 131.89 ± 2.789 and 4.12 ± 0.139 mEq/L respectively. Average ammonia nitrogen in serum was recorded to be 0.42 ± 0.001 mg per cent.

4:1:4 Urine analysis

In general the colour of urine samples was light yellow without any turbidity. Qualitatively all the samples were negative for albumin, glucose, ketone bodies and bile salts.

4:2 Observations on experimentally induced ruminal atony in crossbred calves (untreated group II)

Following intraruminal administration of wheat straw @ 2 kg per 50 kg body weight daily as described under chapter 3:4. Ruminal atony was achieved by 11th day in all the five crossbred calves. Out of five, one

animal died on 11th day, the second on 15th day and third on 17th day of experimental induction of ruminal atony. However two animals recovered miserably and were weak and debilitated by 19th day of experiment.

4:2:1 Clinical observation

The observations on clinical parameters following intraruminal engorgement of wheat bhusa are presented in Table 6.

0 day : Prior to intraruminal administration of wheat straw, all animals were normal and active. The mean body temperature and rumen motility were $39.08 \pm 0.037^{\circ}\text{C}$ and 3.00 ± 0.00 per two minutes respectively. The conjunctival mucous membrane was roseate in colour and eyes were bright in appearance. Both urination and defecation were normal.

1st day : All animals were normal and active. The body temperature was $39.11 \pm 0.041^{\circ}\text{C}$ and rumen motility 3.20 ± 0.200 /two minutes.

3rd day : There was slight increase in rumen motility (3.40 ± 0.245 per two minutes). Two animals had slight anxious look or otherwise they were alright clinically.

5th day : Dullness and restlessness were exhibited by all the animals on 5th day. Abdomen looked slightly distended. The ruminal movements were weak in frequency and amplitude (2.45 ± 0.245 /two minutes). Ruminal contents were foul smelling. The mean body temperature recorded was $38.84 \pm 0.022^{\circ}\text{C}$. The animals passed hard constipated faecal balls.

Table 6. Clinical observations in experimentally induced ruminal atony in crossbred calves (Group II).

Parameters	Day(s) Mean \pm S.E.					
	0	1	3	5	7	9
General condition and appearance	Normal and active	Normal and active	Normal and active	Slight dullness and restlessness	Dullness, depression, constipation suspended rumination and uneasiness.	Complete anorexia depression, reluctance to move, suspended rumination and severe grinding of teeth.
Abdomen	Normal	Normal	Normal	Slightly distended	Gross distension	Over distension
Rumen motility (per two minutes)	3.00 ± 0.00	3.20 ± 0.200	3.40 ± 0.245	2.4 ± 0.245	1.6 ± 0.245	0.8 ± 0.200
Temperature ($^{\circ}$ C)	39.08 ± 0.037	39.11 ± 0.041	39.15 ± 0.035	38.84 ± 0.022	38.80 ± 0.012	38.52 ± 0.041
Mucous membrane	Roseate	Roseate	Roseate	Roseate	Roseate	Slightly congested
No. of animals	5	5	5	5	5	5

contd..

Table 6 contd..

Parameters	Day(s) Mean \pm S.E.				
	11	13	15	17	19
General condition and appearance	Gastrointestinal atony constipation and pelleted faeces. Severe grinding of teeth. Suspended rumination and moderate dehydration. One animal was recumbent with dyspnoea and died.	Animals exhibited ruminal atony and severe impaction of stomach and loss of condition, anorexia and dullness. Constipated dung.	Symptoms as on 13th day except that one animal was recumbent and could not get up and died in the evening.	Animal had loss of condition and indicated stress and anxious behaviour, complete in appetite one animal died.	Animal showed interest in taking fodder. Ruminal movements weak in amplitude and frequency. Loss of condition was evident. Polydypsia was noticed.
Abdomen	Over distended doughy and impacted	Doughy and impacted	Distended	Distended	Slightly distended
Rumen motility (per two minutes)	0.00 ± 0.00	0.00 ± 0.00	0.75 ± 0.250	1.33 ± 0.333	1.50 ± 0.500
Temperature ($^{\circ}\text{C}$)	38.20 ± 0.022	38.25 ± 0.025	38.45 ± 0.137	38.6 ± 0.152	38.75 ± 0.150
Mucous membrane	Congested	Congested	Congested	Pallor	Pallor
No. of animals	5	4	4	3	2

7th day : Marked dullness and depression were noticed in all the animals. Rumination was suspended and the abdomen grossly distended. Animals were uneasy and reluctant to move and passed hard pelleted faeces. The mean body temperature and rumen motility recorded were $38.80 \pm 0.012^{\circ}\text{C}$ and 1.6 ± 0.245 . The rest of observations were similar to 5th day.

9th day : By 9th day, complete anorexia and depression was noticed in all the calves. Severe grinding of teeth was observed on three animals while one animal showed froathy salivation and dyspnoea. Rumen motility was considerably feeble (0.8 ± 0.200 /two minutes). Conjunctival mucous membrane was slightly congested.

11th day : There was complete atony of rumen in all the animals. The rumen appeared doughy and impacted. One animal became recumbent with dyspnoea owing to acute impaction of rumen. The calves exhibited severe grinding of teeth and had mild degree of dehydration which was judged by tenting of skin fold which persisted for 3 seconds. There was severe constipation and the pelleted dung was smeared with mucous and blood streaks. A slight decrease in body temperature ($38.20 \pm 0.022^{\circ}\text{C}$) was noticed.

13th day : Complete ruminal stasis and impaction were present. There was loss of condition and hair coat was rough. The other symptoms were similar to those observed on 11th day. The mucous membrane was injected. The mean body temperature recorded was $38.25 \pm 0.025^{\circ}\text{C}$.

15th day : Clinically all the calves were as on 13th day except one which was recumbent, and died in the evening. The surviving animals had rough hair coat, congested mucous membrane and sunken eyes. Animals were anorectic but had polydypsia.

17th day : The 3rd calf died as the condition progressed clinically the surviving calves were as on 15th day. However there was slight regain in rumen motility (1.33 ± 0.333 /two minutes). The mucous membrane was pallor and mild distension of abdomen was noticed.

19th day : There was regain in rumen motility though weak in amplitude. Animals had desire for fodder and slight rumination was exercised by the calves.

4:2:2 Changes in rumen liquor

4:2:2:1 Physical changes

The observations on physical characteristics of rumen liquor in experimentally induced ruminal atony are presented in Table 7.

Initially rumen fluid was greenish brown in colour which changed to yellowish brown by 3rd day and became dark brown by 9th day and finally turned yellowish on 19th day of experiment. The consistency of rumen fluid was viscous, which became watery by 5th day and remained so till 19th day of observation. Aromatic odour of normal rumen liquor changed to slight putrid by 5th day and was putrid and pungent till 19th day.

Table 7. Physical characteristics of rumen liquor in experimentally induced ruminal atony in crossbred calves (Group II).

Day(s)	Colour	Consistency	Odour	pH	Motility	Concentration	S.A.T.	No. of animals
0	Greenish brown	Viscous	Aromatic	6.88 ±0.052	+++	+++	13.8 ±0.374	5
1	"	"	"	6.75 ±0.055	+++	+++	13.4 ±0.600	5
3	Yellowish brown	"	"	6.86 ±0.076	+++	+++	12.6 ±0.245	5
5	"	Watery	Slightly putrid	7.10* ±0.049	+++	++	14.6 ±0.245	5
7	"	"	Putrid	7.52** ±0.085	++	++	18.2** ±0.490	5
9	Dark brown	"	Putrid	7.89** ±0.044	+	+	30.2** ±0.374	5
11	"	"	"	8.16** ±0.087	+	+	Nil	5
13	"	"	"	8.00** ±0.035	+	+	Nil	4
15	"	"	"	7.91** ±0.032	+	+	Nil	4
17	"	"	"	7.86** ±0.028	+	+	32.33** ±1.453	3
19	Yellowish	"	"	7.79** ±0.095	+	++	32.00** ±0.00	2

* Significant at 5% level of significance.

** Significant at 1% level of significance.

The sedimentation activity time was significantly ($P \leq 0.01$) increased from the initial mean value of 13.8 ± 0.374 minutes to 30.2 ± 0.374 minutes by 9th day. No sedimentation activity was noted from 10th to 16th day. However on 17th day significantly delayed SAT was observed (32.33 ± 1.453 minutes).

There was a gradual reduction in protozoal motility. It was vigorous (+++) on zero day, moderate on 7th day and became sluggish (+) from 11th day of experiment. Rumen protozoal concentration showed the similar trend. It decreased to 10-15 protozoa per LPF (++) by 5th day and consequently few (+) protozoa were seen from 11th to 17th day. However by 19th day protozoal concentration increased (++).

4:2:2:2 Microbial changes

The observations on rumen microbial analysis of experimentally induced ruminal atony in crossbred calves are furnished in Table 8.

Initially rumen protozoal count was $2.78 \pm 0.052 \times 10^5$ per ml of strained rumen liquor which increased nonsignificantly to $2.84 \pm 0.053 \times 10^5$ /ml of SRL on 3rd day. The count decreased significantly from 7th day onwards and was least on 11th day ($1.51 \pm 0.065 \times 10^5$). Subsequently it increased ($1.91 \pm 0.038 \times 10^5$) on 19th day but was significantly low as compared to zero day value.

Table 8. Protozoal examination of rumen liquor in experimentally induced ruminal atony in crossbred calves (Group II).

Day(s)	Total Protozoal count. ($\times 10^7$)	Differential protozoan count % Mean \pm S.E.						No. of animals
		Isotricha	Dasytricha	Entodinium	Diplodinium	Epidinium	Unidentified	
0	2.78 ± 0.052	10.4 ± 0.456	4.2 ± 0.334	63.0 ± 0.632	15.4 ± 0.358	3.4 ± 0.219	3.6 ± 0.455	5
1	2.79 ± 0.041	11.2 ± 0.335	4.6 ± 0.219	62.2 ± 0.522	15.0 ± 0.400	3.4 ± 0.219	3.6 ± 0.219	5
3	2.84 ± 0.053	12.4* ± 0.456	4.4 ± 0.219	63.0 ± 0.632	15.4 ± 0.456	3.2 ± 0.179	1.75* ± 0.216	5
5	2.63 ± 0.060	10.8 ± 0.334	3.6 ± 0.456	64.0 ± 0.632	15.2 ± 0.335	3.6 ± 0.219	2.8 ± 0.178	5
7	2.20* ± 0.030	7.4** ± 0.456	2.2** ± 0.178	65.6* ± 0.536	16.4 ± 0.219	4.0 ± 0.238	4.0 ± 0.283	5
9	1.97** ± 0.030	5.2** ± 0.335	2.0** ± 0.283	67.0** ± 0.238	16.2 ± 0.438	3.8 ± 0.335	5.8** ± 0.335	5
11	1.51** ± 0.065	3.6** ± 0.357	1.33** ± 0.272	67.2** ± 0.521	18.0* ± 0.938	3.6 ± 0.219	6.8** ± 0.521	5
13	1.53** ± 0.042	3.75** ± 0.414	1.00** ± 0.00	68.25** ± 0.545	17.25* ± 0.415	3.25 ± 0.217	6.75** ± 0.960	4
15	1.63** ± 0.060	3.50** ± 0.250	1.33** ± 0.471	68.50** ± 0.559	17.00* ± 0.354	3.5 ± 0.250	6.50** ± 0.346	4
17	1.77** ± 0.96	3.66** ± 0.272	1.66** ± 0.272	67.33** ± 0.720	17.00** ± 0.471	2.66 ± 0.472	7.66** ± 0.272	3
19	1.91** ± 0.038	4.5** ± 0.350	2.0** ± 0.00	68.00** ± 0.00	16.5* ± 0.354	3.00 ± 0.00	6.0** ± 0.00	2

* Significant at 5% level of significance.

**Significant at 1% level of significance.

The differential rumen protozoal pattern of *Isotricha* was $10.4 \pm 0.456\%$ on zero day. It increased significantly ($P \leq 0.05$) on 3rd day ($12.4 \pm 0.456\%$), while from 7th day onwards there was significant ($P \leq 0.01$) decrease and it was least on 15th day ($3.50 \pm 0.250\%$). However no appreciable difference could be noted by 17th and 19th day of observation. The count of *Dasytricha* decreased significantly from 7th day onwards till the end of the experiment. It was lowest on 13th day ($1.00 \pm 0.00\%$) as compared to zero day value ($4.2 \pm 0.334\%$).

Ertodinium species, which were $63.0 \pm 0.632\%$ at the beginning of experiment increased significantly from 7th day onwards and remained higher till 19th day.

Diplodinium species showed a gradual but significant ($P \leq 0.05$) increase on 11th day and remained significantly high throughout the period of the experiment.

Normal *Epidinium* count was $3.4 \pm 0.219\%$ on zero day. It showed least fluctuations during the entire period of experiment.

Unidentified and other species were $3.6 \pm 0.455\%$ on zero day. A significant ($P \leq 0.05$) decrease in their count was noted on 3rd day. However their pattern increased significantly ($P \leq 0.01$) by 9th day and remained significantly high till 19th day of experiment.

Rumen bacterial pattern

Gram stained rumen fluid smears showed predominance of Gram negative bacteria throughout the period of experiment. Rods, coccobacilli and cocci were in majority.

4:2:2:3 Biochemical changes in rumen fluid

The results of biochemical analysis of rumen liquor in animals of group II are presented in Table 9 and Fig. 1, 2 and 3. There was gradual but significant ($P < 0.01$) increase in pH of rumen liquor. It was 7.10 ± 0.049 ; 7.52 ± 0.085 ; 7.89 ± 0.044 and 8.16 ± 0.087 on 5th, 7th, 9th and 11th day respectively. The highest individual rumen pH among the animals of this group was 8.50 on 11th day. From 11th day onwards, nonsignificant decrease in ruminal pH was recorded.

From the average pre-induction value of 12.37 ± 0.575 mg percent, ammonia-nitrogen increased significantly ($P < 0.01$) from 7th day onwards and reached maximum concentration of 23.70 mg per cent on 11th day of the experiment. It remained significantly high even by 19th day.

A significant ($P < 0.01$) decrease was recorded in TVFA concentration from initial mean value of 57.75 ± 2.450 mEq/L to 34.30 ± 1.867 mEq/L on 11th day. Subsequently, it started increasing and reached 44.60 ± 0.717 mEq/L on 19th day of the observation.

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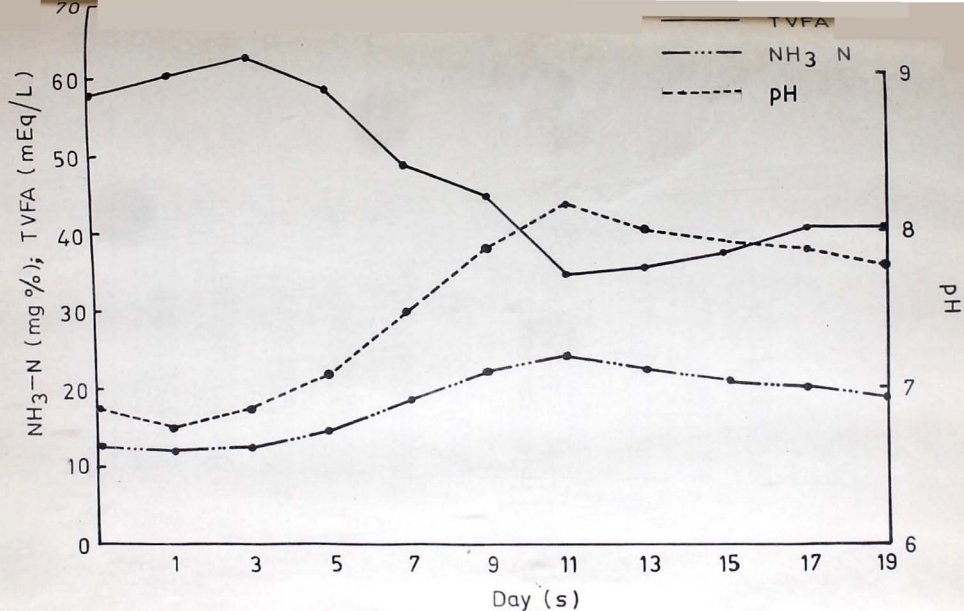


FIG.1. RUMEN FLUID BIOCHEMICAL CHANGES IN EXPERIMENTALLY INDUCED RUMINAL ATONY IN CROSSBRED CALVES (UNTREATED GROUP II)

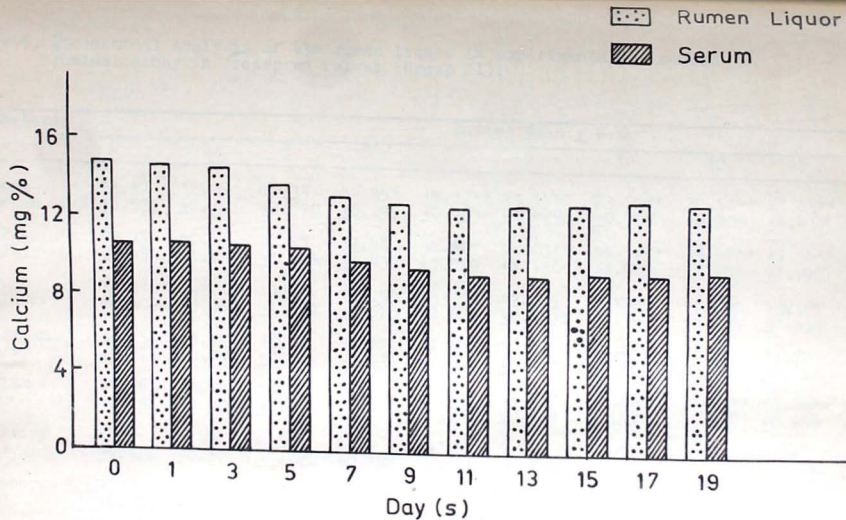


FIG. 2. DEPICTING CALCIUM LEVEL IN RUMEN LIQUOR AND SERUM IN EXPERIMENTALLY INDUCED RUMINAL ATONY IN UNTREATED CALVES (GROUP II)

Table 9. Biochemical analysis of the rumen liquor in experimentally induced ruminal atony in crossbred calves (Group II).

Parameters	Day(s) Mean \pm S.E.										
	0	1	3	5	7	9	11	13	15	17	19
Ammonia nitrogen (mg%)	12.37 ± 0.575	12.06 ± 0.108	12.35 ± 0.590	14.35* ± 0.299	18.17** ± 0.455	21.15** ± 0.425	23.70** ± 0.735	22.50** ± 0.308	21.63** ± 0.437	20.33** ± 0.593	18.62** ± 0.407
T.V.F.A. (mEq/L)	57.75 ± 2.450	60.64 ± 1.872	62.55 ± 1.858	58.80 ± 1.432	48.60* ± 1.709	44.03** ± 1.130	34.30** ± 1.867	35.98** ± 1.969	37.54** ± 1.777	41.18** ± 3.392	40.60** ± 0.717
Sodium (mEq/L)	141.16 ± 3.607	142.15 ± 3.080	139.84 ± 1.687	133.50 ± 2.576	133.91 ± 2.628	132.05 ± 2.473	131.85 ± 2.147	132.06 ± 1.294	132.81 ± 1.631	133.25 ± 1.338	133.27 ± 1.502
Potassium (mEq/L)	32.75 ± 0.812	33.14 ± 0.742	33.00 ± 0.642	33.17 ± 0.634	32.82 ± 0.674	32.19 ± 0.190	31.41 ± 0.863	31.81 ± 0.736	31.72 ± 0.926	32.02 ± 0.577	32.11 ± 0.919
Calcium (mg%)	14.76 ± 0.298	14.47 ± 0.306	14.44 ± 0.711	13.83 ± 0.403	13.04 ± 0.563	12.98* ± 0.688	12.66** ± 0.424	12.86** ± 0.424	12.88** ± 0.248	12.86** ± 0.393	12.90** ± 0.433
Magnesium (mg%)	6.50 ± 0.186	6.44 ± 0.173	6.35 ± 0.220	6.34 ± 0.222	6.18 ± 0.140	6.01 ± 0.146	5.99 ± 0.140	5.91 ± 0.149	5.97 ± 0.150	5.81 ± 0.256	5.91 ± 0.188
Phosphorus (mg%)	9.70 ± 0.223	9.62 ± 0.168	9.71 ± 0.211	9.52 ± 0.248	9.31 ± 0.248	9.29 ± 0.356	8.98 ± 0.256	8.96 ± 0.188	8.91 ± 0.156	8.83 ± 0.292	8.89 ± 0.056
Thiamine (mcg%)	5.82 ± 0.170	5.91 ± 0.147	6.14 ± 0.127	5.71 ± 0.181	4.94 ± 0.229	4.07* ± 0.079	3.88** ± 0.140	3.75** ± 0.174	3.79** ± 0.206	3.81** ± 0.192	3.93** ± 0.017
No. of animals	5	5	5	5	5	5	5	4	4	3	2

* Significant at 5% level of significance.

** Significant at 1% level of significance.

Sodium level did not vary much at any stage of the experiment. It was 131.85 ± 2.147 mEq/L on 11th day as compared to zero day value of 141.16 ± 3.607 mEq/L. The initial concentration of potassium was 32.75 ± 0.812 mEq/L and it followed the similar trend as that of sodium and decreased slightly to 31.41 ± 0.863 mEq/L on 11th day.

There was significant ($P < 0.05$) decrease in rumen calcium level (12.98 ± 0.688 mg per cent) on 9th day as compared to initial mean value of 14.76 ± 0.298 mg per cent. It further dropped significantly ($P < 0.01$) (12.66 ± 0.424) on 11th day. The magnesium concentration of rumen liquor also decreased marginally (5.99 ± 0.140 mg per cent) from the initial mean value of 6.50 ± 0.186 mg per cent on 11th day.

Inorganic phosphorus level decreased gradually throughout the experiment however the decrease was nonsignificant statistically.

Significant ($P < 0.01$) fall in rumen thiamine level was observed in the present study. From the base value of 5.82 ± 0.170 mcg% it lowered to 4.07 ± 0.079 and 3.88 ± 0.140 mcg% on 9th and 11th day respectively. The level of thiamine remained significantly ($P < 0.01$) low till last observation.

4:2:3 Changes in blood/serum

The results of blood/serum biochemical analysis are presented in table 10 and Fig.2 and 3).

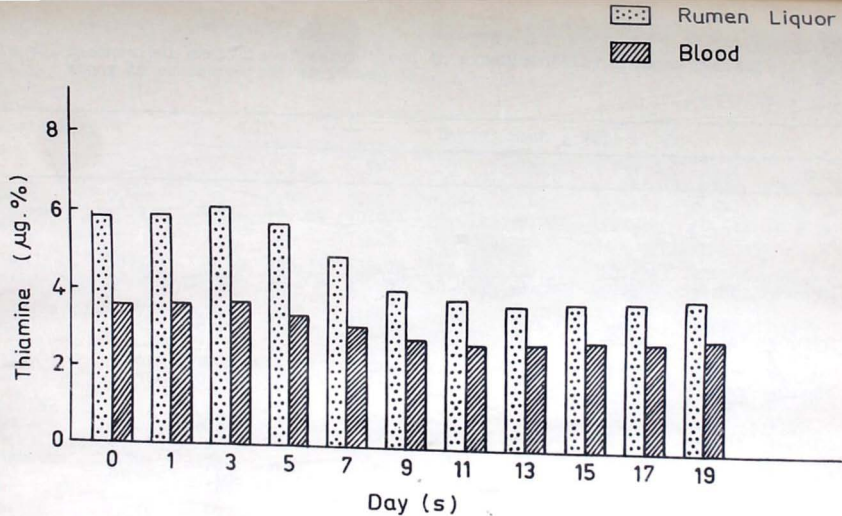


FIG.3. SHOWING LEVEL OF THIAMINE IN RUMEN LIQUOR AND BLOOD OF CROSSBRED CALVES BEFORE AND AFTER INDUCTION OF RUMINAL ATONY (UNTREATED GROUP II)

Table 10. Biochemical analysis of serum/blood in experimentally induced ruminal atony in crossbred calves (Group II).

Parameters	Day(s) Mean \pm S.E.										
	0	1	3	5	7	9	11	13	15	17	19
Ammonia nitrogen (mg%)	0.45 ± 0.038	0.43 ± 0.024	0.42 ± 0.024	0.45 ± 0.021	0.49 ± 0.029	0.49 ± 0.031	0.51 ± 0.038	0.54 ± 0.013	0.54 ± 0.013	0.49 ± 0.024	0.48 ± 0.060
Sodium (mEq/L)	135.24 ± 2.226	134.40 ± 1.908	134.35 ± 2.228	132.76 ± 1.767	131.40 ± 2.743	129.35 ± 2.249	126.79 ± 2.504	128.86 ± 2.223	130.44 ± 1.859	130.25 ± 2.917	131.55 ± 1.273
Potassium (mEq/L)	4.77 ± 0.153	4.71 ± 0.146	4.47 ± 0.172	4.38 ± 0.133	4.39 ± 0.149	4.32 ± 0.167	4.23 ± 0.143	4.29 ± 0.165	4.37 ± 0.187	4.41 ± 0.271	4.42 ± 0.124
Calcium (mg%)	10.62 ± 0.180	10.60 ± 0.182	10.46 ± 0.163	10.22 ± 0.161	9.81 ± 0.303	9.45* ± 0.248	9.11** ± 0.371	9.05** ± 0.362	9.15** ± 0.220	9.21** ± 0.214	9.25** ± 0.250
Magnesium (mg%)	1.91 ± 0.083	1.90 ± 0.038	1.87 ± 0.087	1.86 ± 0.086	1.76 ± 0.098	1.61 ± 0.096	1.60 ± 0.103	1.58 ± 0.115	1.61 ± 0.103	1.69 ± 0.066	1.74 ± 0.071
Phosphorus (mg%)	3.76 ± 0.165	3.73 ± 0.106	3.74 ± 0.111	3.66 ± 0.078	3.61 ± 0.099	3.52 ± 0.101	3.39 ± 0.093	3.41 ± 0.090	3.45 ± 0.081	3.47 ± 0.116	3.56 ± 0.038
Thiamine (mcg%)	3.56 ± 0.089	3.62 ± 0.081	3.65 ± 0.088	3.43 ± 0.089	3.12* ± 0.110	2.84** ± 0.109	2.71** ± 0.084	2.75** ± 0.075	2.79** ± 0.028	2.76** ± 0.065	2.87** ± 0.050
No. of animals	5	5	5	5	5	5	5	4	4	3	2

* Significant at 5% level of significance.

** Significant at 1% level of significance.

Initial concentration of serum ammonia-nitrogen was 0.45 ± 0.038 mg% which increased gradually and non-significantly to 0.54 ± 0.013 mg% on 13th day.

Sodium content of serum decreased to 126.79 ± 2.504 mEq/L on 11th day from the initial value of 135.24 ± 2.226 and thereafter it showed slight increasing trend and reached 131.55 ± 1.273 mEq/L on 19th day. Serum potassium level which was 4.77 ± 0.153 mEq/L on zero day, showed partial and nonsignificant decrease on 11th day to 4.23 ± 0.143 mEq/L.

Calcium level in serum decreased significantly ($P < 0.01$) to 9.11 ± 0.371 mg per cent on 11th day as compared to 10.62 ± 0.180 mg% on zero day and was lowest (9.05 ± 0.362 mg%) on 13th day. It remained significantly ($P < 0.01$) low till 19th day of experiment. The mean magnesium level showed least change from the base value of 1.91 ± 0.083 mg%. It was 1.58 ± 0.115 mg% on 13th day.

The inorganic phosphorus level also showed a decreasing trend from the base value of 3.76 ± 0.165 mg%. However the decrease was statistically nonsignificant.

Thiamine concentration of blood decreased significantly ($P < 0.01$) from the base value of 3.56 ± 0.089 mcg per cent to lowest (2.71 ± 0.084 mcg%) on 11th day. Its concentration in serum remained significantly ($P < 0.01$) low upto 19th day of observation.

4:2:2 Urine analysis in untreated crossbred calves (Group II)

The colour of the urine was light yellow to yellow without turbidity during the entire period of the experiment. Qualitatively albumin, glucose, ketone bodies and bile salts were absent in the urine samples during the experiment.

4:3 Observations on experimentally induced ruminal atony in crossbred calves (Treated group III)

Ruminal atony was induced in all the calves of this group as in group II. The treatment was instituted as soon as complete atony of rumen was noted. Among five animals of this group, one animal died on 11th day and rest four recovered completely. The efficacy of therapy was assessed based on clinical as well as rumen fluid and blood biochemical observations.

4:3:1 Clinical observations

The clinical manifestations at different stages of induction of ruminal atony resembled closely to those observed in animals of group II and are presented in Table 11.

Animals were quite normal and active for first three days with normal urination and defecation. However rumen was slightly ~~more~~ motile on 3rd day (3.20 ± 0.200 per two minutes) as compared to zero day (3.00 ± 0.00). Mucous membrane was roseate in colour and no

Table 11. Clinical observations in experimentally induced ruminal atony in crossbred calves (Group III).

Parameters	Day(s) Mean \pm S.E.					
	0	1	3	5	7	9
General condition and appearance	Normal and active	Normal and active	Normal and active	Dullness, anxious behaviour	Dullness, depression, in appetence and reluctance to move constipation	Suspended rumination and complete anorexia constipation, severe grinding of teeth staining and mild dehydration
Abdomen	Normal	No distension	No distension	Mild distension	Hard and distended	Impacted
Rumen motility (per two minutes)	2.8 ± 0.200	3.00 ± 0.00	3.2 ± 0.200	2.6 ± 0.245	1.8 ± 0.200	0.6 ± 0.245
Body temperature ($^{\circ}\text{C}$)	39.11 ± 0.037	39.11 ± 0.041	39.15 ± 0.035	39.10 ± 0.022	38.91 ± 0.012	38.50 ± 0.041
Mucous membrane	Roseate	Roseate	Roseate	Roseate	Roseate	Slightly congested
No. of animals	5	5	5	5	5	5

contd...

Table 11 contd..

Parameters	Day(s) Mean \pm S.E.						
	11	12	13	14	15	16	17
General condition and appearance	Complete ruminal stasis suspended rumination, anorexia and severe grinding of teeth. There was mild dehydration and depression and constipation	Animal showed urge for taking large quantities of water but refused to eat. Rough hair coat and emaciation	Appetite had improved but animal passed constipated dung.	Animal showed considerable improvement. Normal appetite and gait.	Bright and alert and clinically normal	Normal and active	Normal and active
Abdomen	Impacted and doughy	Slightly distended	Mild distension	No distension	No distension	No distension	No distension
Rumen motility (per two minutes)	Nil	1.25 ± 0.250	1.75 ± 0.250	2.50 ± 0.289	3.00 ± 0.00	2.75 ± 0.250	3.00 ± 0.00
Body temperature ($^{\circ}$ C)	38.10 ± 0.022	38.42 ± 0.020	38.94 ± 0.025	39.11 ± 0.035	39.12 ± 0.031	39.12 ± 0.031	39.10 ± 0.022
Mucous membrane	Congested	Congested	Congested	Congested	Normal	Normal	Roseate
No. of animals	5	4	4	4	4	4	4

abnormal distension was seen. However on 3rd day one animal exhibited uneasiness and had polydipsia.

5th day : All animals showed anxious look but were dull. Constipation was seen in three animals. The rumen motility was 2.6 ± 0.245 per two minutes and the body temperature being $39.10 \pm 0.032^{\circ}\text{C}$.

7th day : Marked dullness and depression were noted in all the animals. Animals exhibited uneasiness and reluctance to move about. Animals showed least interest in surroundings. Rumen was hypomotile (1.8 ± 0.200 per two minutes). Mean body temperature recorded as 38.91°C .

9th day : All animals were completely anorectic and preferred to lie down. Severe grinding of teeth by all the animals was observed. One animal showed frothy salivation, mucous nasal discharge and dyspnoea. All the animals passed hard, pelleted faeces. The mean rumen motility recorded was 0.6 ± 0.245 per two minutes. The mucous membrane was injected and rumen impacted and hard to touch.

11th day : Observations were similar to those recorded on 9th day except that complete ruminal stasis was observed in all the animals. There was mild dehydration as skin fold remained elevated for 3.0 seconds. Animals passed pelleted dung smeared with mucous and blood. One animal was recumbent with severe dyspnoea and died in the afternoon. Rest of the animals had sunken eyes, loss of

conditions and anuria. Abdomen was doughy and impacted.

12th day : Animals consumed large quantity of water. They were weak and had rough hair coat. Severe congestion of conjunctival mucous membrane was noted in all the animals. The rumen was atonic. Animals refused to eat even the green fodder.

13th day : All the animals were able to walk and rumen motility had reappeared (1.75 ± 0.250) per two minutes. Urination was normal but there was no improvement in consistency of the dung.

14th day : Animals showed desire for feed and water and were clinically normal. The rumen motility was 3.0 ± 0.00 per two minutes. Dung was of normal consistency. Mucous membrane was roseate in colour and general condition had improved.

16th and 17th day : Clinically all the animals were normal. Urination and defecation were normal.

4:3:2 Changes in rumen liquor

4:3:2:1 Physical changes

The results of physical examination of rumen liquor in animals of group III are presented in Table 12.

Initially the colour of rumen liquor which was greenish brown, turned yellowish on 5th day and to dark brown on 9th day. On 13th day it became yellowish brown

Table 12. Physical characteristics of rumen liquor in experimentally induced ruminal atony crossbred calves (Group III).

Day(s)	Colour	Consistency	Odour	pH	Motility	Concentration	S.A.T. (minutes)	No. of animals
0	Greenish brown	Viscous	Aromatic	6.83 ±0.078	+++	+++	15.00 ±0.250	5
1	"	"	"	6.82 ±0.066	+++	+++	13.8 ±0.374	5
3	"	"	"	6.83 ±0.086	+++	+++	12.6 ±0.245	5
5	Yellowish	Watery	Slightly putrid	7.08 ±0.071	+++	+++	20.00 ±0.250	5
7	"	"	"	7.66** ±0.149	++	++	22.25 ±0.218	5
9	Dark brown	"	Putrid	8.01** ±0.085	+	+	Nil	5
11	"	"	"	8.14** ±0.064	+	+	Nil	5
12	"	"	"	7.77** ±0.062	+	+	Nil	4
13	Yellowish brown	"	Slightly putrid	7.26* ±0.090	++	+	18.1 ±0.453	4
14	Greenish brown	Viscous	Aromatic	6.88 ±0.105	+++	++	18.2 ±0.310	4
15	"	"	"	6.85 ±0.079	+++	++	15.0 ±0.200	4
16	"	"	"	6.88 ±0.042	+++	++	15.10 ±0.310	4
17	"	"	"	6.85 ±0.060	+++	++	15.00 ±0.200	4

* Significant at 5% level of significance.

** Significant at 1% level of significance.

and regained its normal colour (greenish brown) on 14th day of observation.

Viscous consistency of normal rumen liquor became watery on 5th day engorgement with wheat straw. Regain in its viscous consistency was noted on 14th day, following treatment.

From aromatic odour of rumen liquor, it changed to slightly putrid on 5th day and offensive on 9th day of the experiment. Following therapy the odour changed to aromatic by 15th day.

Sedimentation activity time of rumen liquor which was 15.00 ± 0.250 minutes, increased to 22.25 ± 0.218 minutes on 7th day. The activity was nil on 9th, 11th and 12th day of the experiment. The sedimentation activity reappeared on 13th day following treatment and by 15th day it was almost normal.

From vigorous (+++) motility, the protozoa became sluggish (+) on 9th day of wheat straw engorgement. After receiving treatment it became moderate (+++) on 13th day and from 14th day it was vigorous again. Initial concentration of protozoa was above 30 per LPF (+++) which became (+) less than 10 per LPF on 9th day. Even after treatment the protozoal concentration did not reach its normal strength.

4:3:2:2 Microbial changes

The observations on microbial changes in the rumen liquor of calves which received therapy after experimental induction of ruminal atony are presented in Table 13.

Total protozoal count of rumen liquor in animals of this group was $2.73 \pm 0.075 \times 10^5$ per ml of SRL at '0' day. Only slight (nonsignificant) increase was noted on first and third day (2.76 ± 0.087) and (2.87 ± 0.066). Thereafter the count decreased gradually and was lowest (1.54 ± 0.072) on 11th day. Following treatment, the protozoal count remained significantly ($P < 0.01$) low upto 15th day. However on 17th day the count was $2.70 \pm 0.77 \times 10^5$ per ml of rumen fluid.

The differential protozoal count revealed increased percentage of *Isotricha* species upto 3rd day. Thereafter the percentage of *Isotricha* declined gradually. It was significantly ($P < 0.01$) low on 11th day ($3.4 \pm 0.245\%$). After therapy *Isotricha* species improved gradually but were significantly ($P < 0.05$) low till 16th day. On 17th day normal percentage (9.25 ± 0.629) was recorded.

Dasytricha also decreased gradually as compared to zero day value of $4.0 \pm 0.316\%$. The lowest percentage (1.6 ± 0.245) was recorded on 11th day. After therapy their count gradually increased and it was $3.75 \pm 0.250\%$ on 17th day which was nearly equal to zero day value.

Table 13. Protozoal examination of rumen liquor in experimentally induced ruminal atony in crossbred calves (Group III).

Day(s)	Total protozoal count ($\times 10^5$)	Differential protozoan counts% Mean \pm S.E.						No. of animals
		Iso-tricha	Dasy-tricha	Ento-dinium	Diplo-dinium	Epidi-nium	Unidenti-fied	
0	2.73 ± 0.075	11.2 ± 0.0583	4.0 ± 0.316	60.6 ± 0.678	16.6 ± 0.538	4.0 ± 0.316	3.6 ± 0.245	5
1	2.76 ± 0.087	11.6 ± 0.748	4.6 ± 0.400	60.4 ± 0.510	16.2 ± 0.374	3.4 ± 0.245	3.8 ± 0.663	5
3	2.87 ± 0.066	12.0 ± 0.548	4.8 ± 0.374	61.2 ± 0.374	16.0 ± 0.447	3.6 ± 0.245	2.4 ± 0.678	5
5	2.45* ± 0.061	10.6 ± 0.510	3.6 ± 0.245	63.0 ± 0.707	16.0 ± 0.447	3.4 ± 0.400	3.4 ± 0.872	5
7	2.21** ± 0.071	8.4* ± 0.509	2.6* ± 0.245	64.8** ± 0.374	16.0 ± 0.447	3.6 ± 0.245	4.6 ± 0.400	5
9	1.94** ± 0.052	5.0** ± 0.316	2.0** ± 0.316	66.8** ± 0.663	16.6 ± 0.510	3.4 ± 0.245	6.2** ± 0.583	5
11	1.54** ± 0.072	3.4** ± 0.245	1.6** ± 0.245	67.6** ± 0.510	16.8 ± 0.583	4.0 ± 0.316	6.6** ± 0.510	5
12	1.65** ± 0.048	3.5** ± 0.289	1.75** ± 0.250	67.0** ± 0.913	16.25 ± 0.629	3.75 ± 0.250	7.5** ± 0.866	4
13	1.95** ± 0.055	4.75** ± 0.428	2.50* ± 0.289	65.25* ± 1.109	17.25 ± 0.479	4.0 ± 0.408	6.25** ± 0.750	4
14	2.35* ± 0.078	6.25** ± 0.479	2.75* ± 0.250	64.0* ± 0.816	17.0 ± 0.408	3.75 ± 0.250	6.25** ± 0.479	4
15	2.46* ± 0.069	7.00* ± 0.408	3.25 ± 0.250	62.25 ± 0.854	17.0 ± 0.408	4.25 ± 0.250	6.25** ± 0.479	4
16	2.56 ± 0.046	8.75 ± 0.479	3.50 ± 0.289	61.25 ± 0.479	17.25 ± 0.479	3.75 ± 0.250	5.50* ± 0.500	4
17	2.60 ± 0.077	9.25 ± 0.629	3.75 ± 0.250	61.50 ± 0.645	16.75 ± 0.479	4.25 ± 0.250	5.50* ± 0.345	4

* Significant at 5% level of significance.

** Significant at 1% level of significance.

Significant ($P \leq 0.01$) increase in Entodinium species was recorded from 7th day onwards as compared to the base value of ($60.6 \pm 0.678\%$). After therapy their percentage decreased gradually and reached almost zero day value on 16th day ($61.25 \pm 0.479\%$).

There were only marginal fluctuation in the Diplodinium species throughout the period of experiment and values lied between 16.0 to 17.25%. Zero day, Epidinium count was $4.0 \pm 0.316\%$. The count did not show such alteration during the entire period of study.

A significant ($P \leq 0.01$) increase was recorded in unidentified protozoa from 9th day onwards. It was $6.6 \pm 0.510\%$ on 11th day. Even on 17th day the count ($5.50 \pm 0.645\%$) was significantly ($P \leq 0.05$) higher as compared to zero day value.

Bacterial pattern

Gram stained rumen fluid smears revealed the predominance of Gram negative bacteria. However from 15th day onwards some Gram positive rods and cocci were also detected.

4:3:2:3 Biochemical changes in the rumen liquor of treated calves (group III).

Rumen fluid biochemical changes in the calves of group III have been presented in Table 14 and Fig.4,5 and 6.

The mean rumen fluid pH increased significantly ($P \leq 0.01$) from 6.83 ± 0.078 to 8.14 ± 0.064 on 11th day,

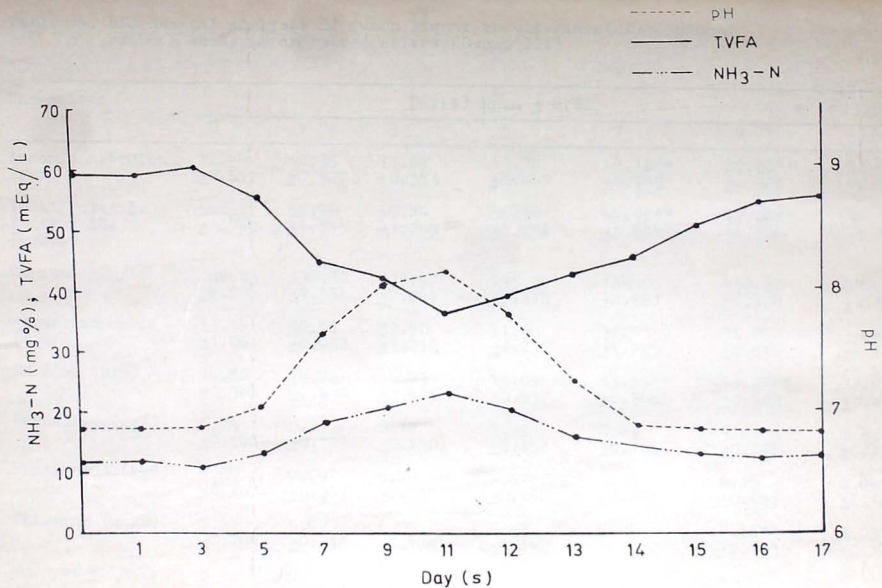


FIG. 4. RUMEN FLUID BIOCHEMICAL CHANGES IN EXPERIMENTALLY INDUCED RUMINAL ATONY IN CROSSBRED CALVES GIVEN THERAPY (GROUP III)

Table 14. Biochemical analysis of rumen liquor in experimentally induced ruminal atony in crossbred calves (Group III)

Parameters	Day(s) Mean \pm S.E.						
	0	1	3	5	7	9	11
Ammonia nitrogen (mg%)	12.44 ± 0.511	12.35 ± 0.327	11.89 ± 0.353	13.78 ± 0.447	18.18** ± 0.595	20.80** ± 0.326	22.95** ± 0.658
Total volatile fatty acids (mEq/L)	59.31 ± 1.740	58.70 ± 1.715	60.14 ± 1.747	56.54 ± 1.236	44.55** ± 1.684	42.06** ± 1.504	36.54** ± 1.298
Sodium (mEq/L)	137.03 ± 2.786	136.60 ± 1.554	135.97 ± 2.891	135.39 ± 1.872	134.05 ± 2.281	132.30 ± 1.928	130.87 ± 1.516
Potassium (mEq/L)	33.17 ± 1.061	32.91 ± 0.932	32.50 ± 1.212	31.98 ± 1.330	31.56 ± 1.229	31.05 ± 0.814	30.85 ± 1.601
Calcium (mg%)	14.86 ± 0.362	14.76 ± 0.438	14.43 ± 0.405	13.89 ± 0.437	13.19* ± 0.346	13.07** ± 0.302	12.83** ± 0.420
Magnesium (mg%)	6.24 ± 0.198	6.21 ± 0.173	6.12 ± 0.110	5.99 ± 0.152	5.89 ± 0.166	5.82 ± 0.176	5.80 ± 0.179
Phosphorus (mg%)	9.80 ± 0.307	9.79 ± 0.261	9.75 ± 0.237	9.57 ± 0.250	9.45 ± 0.251	9.16 ± 0.085	8.94 ± 0.166
Thiamine (mcg%)	5.92 ± 0.200	5.97 ± 0.218	6.09 ± 0.195	5.87 ± 0.197	5.04 ± 0.312	4.16** ± 0.163	3.93** ± 0.125
No. of animals	5	5	5	5	5	5	5

contd...

Table 14 contd..

Parameters	Day(s) Mean \pm S.E.					
	12	13	14	15	16	17
Ammonia nitrogen (mg%)	20.52** ± 0.416	16.22** ± 0.637	14.10 ± 0.602	13.15 ± 0.517	12.80 ± 0.420	12.59 ± 0.333
Total volatile fatty acids (mEq/L)	38.43** ± 1.331	42.26** ± 1.241	45.43** ± 1.608	50.09* ± 1.875	53.06 ± 1.620	53.53 ± 1.347
Sodium (mEq/L)	131.26 ± 2.323	133.66 ± 1.938	135.66 ± 1.396	136.29 ± 1.745	136.62 ± 1.677	137.73 ± 2.015
Potassium (mEq/L)	30.00 ± 0.434	30.46 ± 0.578	31.10 ± 0.424	31.45 ± 1.250	32.19 ± 1.196	32.97 ± 1.845
Calcium (mg%)	12.81** ± 0.180	13.12* ± 0.368	13.05* ± 0.270	13.33* ± 0.432	13.38 ± 0.191	13.45 ± 0.190
Magnesium (mg%)	5.88 ± 0.169	5.84 ± 0.179	5.97 ± 0.128	6.02 ± 0.191	6.06 ± 0.186	6.15 ± 0.144
Phosphorus (mg%)	8.98 ± 0.172	9.03 ± 0.156	9.10 ± 0.167	9.12 ± 0.198	9.15 ± 0.206	9.21 ± 0.164
Thiamine (mcg%)	4.05** ± 0.077	4.12** ± 0.081	4.23** ± 0.081	4.41** ± 0.176	4.68** ± 0.186	5.08* ± 0.182
No. of animals	4	4	4	4	4	4

* Significant at 5% level of significance

** Significant at 1% level of significance

the day of commencement of therapy. After treatment the pH decreased and it was 6.85 ± 0.105 on 15th day.

Ammonia nitrogen concentration of rumen fluid increased significantly from 12.44 ± 0.511 mg% on zero day to 22.95 ± 0.685 on 11th day. Following treatment it declined gradually. The concentration recorded on 17th day was 12.59 ± 0.333 mg% which was very near to zero day value.

A gradual and significant decrease (36.54 ± 0.685) was observed in the concentration of total volatile fatty acids on 11th day as compared to base value of 59.31 ± 1.740 mEq/L. After commencement of therapy, the concentration of TVFA increased gradually but remained significantly low ($P < 0.05$) till 15th day. Even on 17th day, TVFA concentration was low (53.53 ± 1.347) as compared to base value.

The average values of sodium and potassium in rumen liquor varied between 137.03 ± 2.786 to 131.26 ± 2.323 mEq/L and 33.17 ± 1.061 to 30.00 ± 0.434 mEq/L respectively, during entire period of experiment.

A gradual but significant ($P < 0.01$) decrease (12.83 ± 0.420 mg%) was observed in the mean value of calcium concentration of rumen fluid on 11th day as compared to base value of 14.86 ± 0.362 mg%. After therapy, the calcium concentration remained significantly ($P < 0.05$) low till 15th day as compared to zero day value.

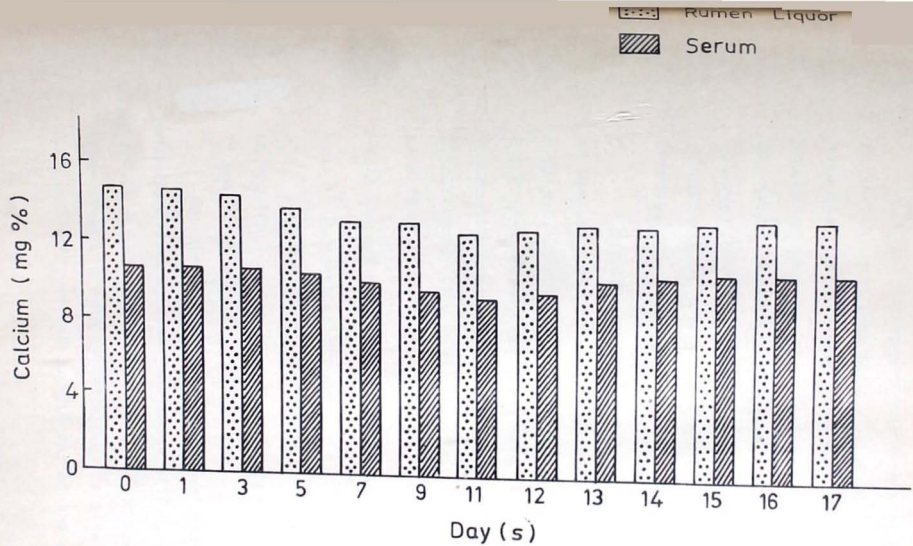


FIG. 5. GRAPH DEPICTING CALCIUM CONCENTRATION IN RUMEN LIQUOR AND SERUM IN EXPERIMENTALLY INDUCED RUMINAL ATONY IN TREATED CROSSBRED CALVES (GROUP III)

Slight but gradual decrease was observed in magnesium level from 6.24 ± 0.198 mg% to 5.8 ± 0.166 mg% on 11th day. After therapy, the magnesium concentration increased gradually but remained low as compared to zero day value. Inorganic phosphorus level of rumen liquor followed the trend of magnesium. It decreased from 9.80 ± 0.307 mg% to 8.94 ± 0.166 mg% on 11th day, which improved after treatment.

A significant ($P < 0.01$) decrease was recorded in ruminal thiamine concentration. It decreased to 3.93 ± 0.125 mcg% on 11th day as compared to 5.92 ± 0.200 mcg% on zero day. Following therapy the rumen thiamine concentration increased gradually but remained significantly ($P < 0.05$) low even on 17th day of experiment.

4:3:3 Changes in blood/serum in treated group.

4:3:3:1 Biochemical changes

The results of biochemical changes in blood/serum in crossbred calves of group III are depicted in table 15 and Fig.5 and 6.

The serum ammonia nitrogen concentration increased non-significantly from base value of 0.42 ± 0.031 mg% to 0.52 ± 0.030 mg% on 11th day. After treatment the concentration gradually decreased. A level of 0.42 ± 0.013 mg% was recorded on 17th day of the experiment.

Table 15. Biochemical analysis of blood/serum in experimentally induced ruminal atony in crossbred calves (Group III).

Parameters	Day(s) Mean \pm S.E.						
	0	1	3	5	7	9	11
Ammonia nitrogen (mg%)	0.42 ± 0.031	0.43 ± 0.030	0.41 ± 0.028	0.43 ± 0.018	0.45 ± 0.030	0.51 ± 0.057	0.52 ± 0.030
Sodium (mEq/L)	134.55 ± 1.597	133.08 ± 1.454	131.83 ± 1.522	131.49 ± 1.731	130.78 ± 1.673	130.06 ± 2.322	128.66 ± 2.102
Potassium (mEq/L)	4.64 ± 0.101	4.62 ± 0.112	4.53 ± 0.057	4.48 ± 0.079	4.41 ± 0.069	4.35 ± 0.061	4.22 ± 0.083
Calcium (mg%)	10.71 ± 0.209	10.68 ± 0.223	10.61 ± 0.232	10.46 ± 0.226	10.01 ± 0.215	9.62* ± 0.184	0.25** ± 0.176
Magnesium (mg%)	1.93 ± 0.057	1.94 ± 0.038	1.86 ± 0.085	1.82 ± 0.078	1.74 ± 0.083	1.68 ± 0.094	1.61 ± 0.080
Phosphorus (mg%)	3.79 ± 0.145	3.72 ± 0.141	3.71 ± 0.147	3.73 ± 0.137	3.67 ± 0.129	3.55 ± 0.122	3.48 ± 0.079
Thiamine (mcg%)	3.50 ± 0.124	3.52 ± 0.109	3.59 ± 0.121	3.45 ± 0.075	3.08* ± 0.069	2.89** ± 0.089	2.71** ± 0.107
No. of animals	5	5	5	5	5	5	5

contd..

Table 15 contd..

Parameters	Day(s) Mean \pm S.E.					
	12	13	14	15	16	17
Ammonia nitrogen (mg%)	0.48 ± 0.021	0.43 ± 0.023	0.41 ± 0.021	0.41 ± 0.019	0.42 ± 0.018	0.42 ± 0.013
Sodium (mEq/L)	132.06 ± 1.984	133.13 ± 1.161	133.38 ± 1.319	134.15 ± 1.448	133.78 ± 0.937	134.06 ± 0.855
Potassium (mEq/L)	4.31 ± 0.072	4.28 ± 0.066	4.24 ± 0.048	4.34 ± 0.043	4.46 ± 0.072	4.48 ± 0.092
Calcium (mg%)	9.67* ± 0.126	10.19 ± 0.210	10.44 ± 0.236	10.54 ± 0.268	10.63 ± 0.289	10.60 ± 0.273
Magnesium (mg%)	1.72 ± 0.072	1.84 ± 0.056	1.91 ± 0.061	1.92 ± 0.062	1.94 ± 0.065	1.95 ± 0.055
Phosphorus (mg%)	3.51 ± 0.107	3.62 ± 0.082	3.63 ± 0.087	3.65 ± 0.071	3.68 ± 0.062	3.64 ± 0.080
Thiamine (mcg%)	2.85** ± 0.067	3.10* ± 0.064	3.29 ± 0.045	3.37 ± 0.022	3.42 ± 0.044	3.49 ± 0.049
No. of animals	4	4	4	4	4	4

* Significant at 5% level of significance.

** Significant at 1% level of significance.

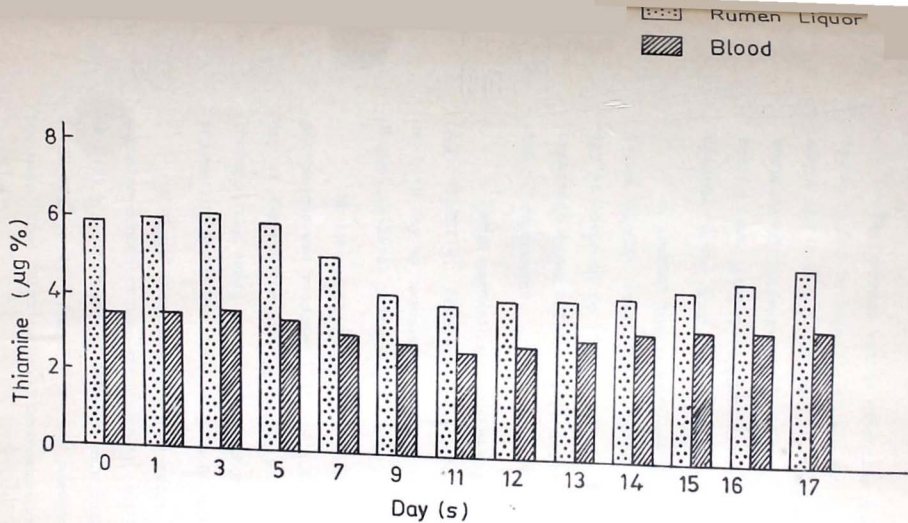


FIG. 6. COMPARATIVE VALUES OF THIAMINE IN RUMEN LIQUOR AND BLOOD IN EXPERIMENTALLY INDUCED RUMINAL ATONY IN CROSSBRED CALVES GIVEN THERAPY (GROUP III)

The average values of sodium and potassium in serum varied between 134.55 ± 1.597 mEq/L to 128.66 ± 2.102 mEq/L and 4.64 ± 0.101 mEq/L to 4.22 ± 0.083 mEq/L respectively throughout the period of experiment. The sodium level increased gradually post-therapy but was slightly lower than base value even on 17th day.

A gradual but significant ($P < 0.01$) decrease in serum calcium level (9.25 ± 0.176 mg%) was noted on 11th day as compared to 10.71 ± 0.209 mg% on zero day. After treatment serum calcium level improved to normal by 14th day of experiment.

Serum magnesium concentration did not vary significantly. However it decreased to 1.61 ± 0.080 mg% on 11th day as compared to 1.93 ± 0.057 mg% on zero day. Magnesium level increased markedly following treatment.

No appreciable change in level of serum inorganic phosphorus was recorded. It was 3.48 ± 0.079 mg% on 11th day as compared to 3.79 ± 0.145 mg% on zero day. After therapy, the serum inorganic phosphorus level showed increasing trend and was 3.64 ± 0.080 mg% on 17th day.

A significant ($P < 0.01$) decrease in mean blood thiamine concentration was observed, the value was as low as 2.71 ± 0.107 mcg% on 11th day when compared to 3.50 ± 0.124 mcg% on zero day. After the commencement of therapy the mean blood thiamine level increased gradually and

reached a concentration of 3.49 ± 0.049 mcg% on 17th day of the experiment.

4:3:4 Urine analysis in treated crossbred calves of group III.

The colour of urine was light yellow to yellow without any turbidity during the entire period. Qualitatively albumin, glucose, ketone and bile salts were absent in the urine samples during the experiment.

5:0 DISCUSSION

Rumen motility is considered as an index of digestive function in cattle. Microbial digestion is unique and important in bovines, because ruminal fauna play a major role in digestion of feed materials. In ruminants, ruminal stasis is the most common clinical problem encountered in the field, when the cattle are exclusively maintained on coarse and dry roughage during the lean periods. There are marked alterations in the rumen microbial population leading to poor and abnormal fermentation process. These changes in the rumen subsequently bring about alterations in certain biochemical constituents of blood (Dunlop, 1972; Slyter, 1976; and Prasad, 1979) causing poor health, low production and even loss of animals.

The clinical norms of the healthy calves in respect of general condition and appearance, rumen motility, body temperature and mucous membrane examined at 48 hour intervals were comparable to those reported by Rosenberger (1979) and Blood *et al.* (1983). Minor fluctuations in these observations were attributable to the age, breed, managemental and climatic factors.

Normal colour, consistency and odour of rumen liquor were observed as greenish brown, viscous and aromatic respectively which were similar to those reported by Misra and Tripathy (1963), Misra *et al.* (1972a), Dash and Misra (1972) and Karunanidhi (1982). The variation in the colour of rumen liquor was comparable to Misra

et al. (1972b) and Karunanidhi (1982) which could be due to the type of fodder fed to the animals. The provision of green fodder to the animals resulted in greenish-brown colour (Alonso, 1979). Vigorous motility and abundant concentration of rumen protozoa were comparable with those reported by Misra et al. (1972b) and Karunanidhi (1982).

The mean sedimentation activity time of rumen liquor (13.60 minutes) was close to the recording of (12.8 minutes) by Misra et al. (1972b). Ingredients of ration fed and time of collection have direct influence on SAT (Rosenberger, 1979) which may account for the wide difference observed in comparison to the value described by Blood et al. (1983).

Both the quality and quantity of feed and frequency of feeding have been known to affect the rumen protozoal concentration (Hungate, 1966; Swenson, 1970). In the present study total protozoan count was $2.65 \pm 0.119 \times 10^5$ per ml of SRL while the differential counts of Isotricha, Dasytricha, Entodinium, Diplodinium, Epidinium and unidentified were 10.76 ± 0.331 , 3.92 ± 0.383 , 62.44 ± 0.924 , 15.64 ± 0.445 , 3.8 ± 0.379 and $3.84 \pm 0.223\%$ respectively. Significant differences in counts as well as differential pattern of rumen protozoa could be due to different feeding regimens (Moir, 1966; Misra et al., 1972a,b). In the

present experiment, predominance of Gram negative bacteria was observed.

The normal pH of rumen liquor in the present experiment ranged between 6.79 ± 0.071 and 7.00 ± 0.061 with an average of 6.89 ± 0.035 , coincides with the reports of Ahrens (1967); Joshi and Misra (1975); Nauriyal (1975); Choudhuri et al. (1981) and Karunanidhi (1982) in calves. The variations observed in pH of normal rumen liquor was largely due to quantity of feed provided and time of sampling.

Rumen ammonia nitrogen level of healthy crossbred calves was 12.81 ± 0.323 mg%, which simulates the findings of Swenson (1970), Prasad (1972) and Allikutty (1981). The level of TVFA observed in the animals of this group was 62.25 ± 1.409 mEq/L, which is comparable with the findings of Sethuraman (1976) and Karunanidhi (1982).

Sodium and potassium concentrations in rumen liquor were similar to the findings of Choudhuri et al. (1981) and Karunanidhi (1982). The levels of calcium, magnesium and inorganic phosphorus in rumen liquor were 14.54 ± 0.492 ; 5.89 ± 0.300 and 8.67 ± 0.215 mg% respectively which are in accordance with findings of Nauriyal (1975).

The normal thiamine content of rumen liquor was observed to be 5.97 ± 0.108 mcg% which is slightly lower

than the values recorded by Gupta et al. (1976); Sinha (1981) and Karunanidhi (1982). The difference might be attributed to type of diet and time of sampling.

The serum ammonia nitrogen level in the present experiment was in agreement with findings of Mayan and Merilan (1976) and Karunanidhi (1982).

Serum sodium and potassium concentrations resembled closely to those of Choudhuri et al. (1981); Sandha (1980) and Sinha (1981). The mean values of serum calcium, magnesium and inorganic phosphorus were 10.24 ± 0.251 , 2.00 ± 0.057 and 3.90 ± 0.064 mg% respectively which were in accordance with the findings of Nauriyal (1975); Shetty et al. (1977) and Sethuraman and Rathor (1979).

The average blood thiamine concentration (3.66 ± 0.992 mcg%) in the present study was similar to the reports of Joshi and Prasad (1975); Gupta et al. (1976) and Karunanidhi (1982) in cattle.

Ruminal atony in cattle and buffaloes has been induced experimentally by exclusive feeding of paddy straw (Misra and Tripathy, 1963). Excessive feeding of coarse roughages during hot and dry season resulted in ruminal atony and subsequently dysfunctions (Joshi and Misra, 1974). In the present experiment intraruminal feeding of wheat straw was done in calves and characteristic

clinical symptoms of ruminal stasis were observed within 9-11 days of feeding. The early onset of the condition in the present work as compared to observation of Misra and Tripathy (1963) might be due to the wheat straw engorgement through rumen fistula. Clinically all the calves had anorexia, constipated and pelleted faeces, dullness, incoordination, reluctance to move, severe grinding of teeth, doughy and impacted rumen, gradual loss of condition and rough hair coat which were the main symptoms of digestive upset. These symptoms were in accordance with observation of Misra and Tripathy (1963); Joshi and Misra (1974) and Prasad (1979). Further three calves died of this condition on 11th, 15th and 17th day of experiment.

Slight increase in rumen motility was recorded initially and thereafter complete stasis was noted on 11th day of the experiment. The irritating effect of fibrous roughage on rumen epithelium could have resulted in hypermotility of the rumen initially while the complete engorgement caused over-stretching leading to ruminal stasis. Hypocalcaemia (vide infra) might also be a contributing factor for ruminal stasis as reported by Prasad et al. (1972) and Daniel (1983). Additionally factors like absorption of toxic amines and amides (Hoflund and Hedstrom, 1948), high butyrate level (Ash, 1959), variations in rumen pH (Dave, 1980;

Karunanidhi, 1982) have been reported to cause ruminal stasis.

Congestion of visible mucous membranes and subnormal body temperature might have been the outcome of venous congestion due to reduction in cardiac output owing to weakness.

Hard, pelleted, mucous and blood coated faeces with foul odour was observed in all the animals from 7th day onwards. Probably this was the outcome of reduced peristalsis and damage caused to mucous membrane of lower alimentary tract by coarse wheat straw.

Dehydration characterised by reduced skin elasticity and sunken eyes, might be due to withdrawal of fluid from vascular compartments into the rumen. Huber (1976) reported anuria in sheep during dehydration because of reduced blood flow to kidneys. Oligouria was recorded in the present experiment. Severe depression and lateral recumbency in one animal on 11th day might be due to absorption of toxins from rumen into the blood and low level of thiamine (Sinha, 1981).

The dark brown colouration of rumen liquor with watery consistency and putrid odour observed in animals of group II was in accordance with observations of Misra and Tripathy (1963) and Misra et al. (1972a) in cattle.

The SAT of rumen liquor increased significantly and it was nil on 11th day. A shift in the pattern of ruminal fauna and poor microbial concentration might have been responsible for increased SAT. This observation agrees with the findings of Rosenberger (1979). Both motility and concentration of rumen protozoa decreased considerably in calves, engorged with wheat straw. Only few protozoa were encountered per LPF. Entodiniomorphs shared the major percentage among the protozoa and there was significant increase in differential counts of Entodinium, Diplodinium and unidentified types of protozoa. Considerable decrease in differential count of Holotrichs was recorded. The disappearance of Holotrichs could be attributed to the absence of favourable substrate and high pH, while as Entodiniomorphs survived owing to their pH tolerance. This finding simulates with the observations of Moir (1959) and Misra and Tripathy (1963), who recorded the predominance of small and large non-ciliate protozoa in cattle maintained exclusively on paddy straw.

Total protozoal counts decreased significantly in all the animals by 9th day, the decrease was probably due to the poor quality of feed, ruminal stasis and unfavourable pH in the pauch. Nauriyal (1975); Alikutty (1981) and Karunanidhi (1982) reported similar observations in calves suffering from ruminal

dysfunction. Rumen fluid smears revealed predominance of Gram negative bacteria which is in accordance with the findings of Hungate (1966). In experimental alkalosis in bovines predominance of Gram negative bacteria has been reported by Sethuraman and Rathor (1980).

A gradual but significant ($P < 0.01$) increase in pH of rumen fluid was recorded during exclusive feeding of wheat straw in all the calves. The finding agrees with the observations of Misra and Tripathy (1963) and Bartley *et al.* (1976). The increase in pH might be due to increased saliva production in order to lubricate the dry, coarse wheat straw. Additionally the putrefaction of ingesta due to ruminal stasis and also production of large quantities of ammonia might have increased the rumen fluid pH. The high ruminal pH had deleterious effect on rumen protozoal population (*vide supra*).

Ammonia nitrogen level of rumen liquor increased significantly ($P < 0.01$) to 23.70 ± 0.735 mg% on 11th day. Putrefaction of ingesta inside rumen due to ruminal atony might have caused increase in production of ammonia. Moreover, alkaline pH of rumen helped in absorption of ammonium ions. Decreased TVFA concentration due to poor microbial activity was also responsible for increase in ammonia nitrogen concentration, since a negative correlation between TVFA and $\text{NH}_3\text{-N}$ has been

reported (Prasad 1977). The present finding is in agreement with those of Joshi and Ludri (1970); Prasad et al. (1972); Joshi and Misra (1975) and Sethuraman (1976).

In rumen, the microbial fermentation of carbohydrates results in formation of acetic acid, propionic acid, butyric acid, valeric acid and other long chain fatty acids. These volatile fatty acids which are the by-products of microbial degradation of feeds, serve the main source of energy for the host animal. In the present experiment decreased TVFA level was observed by 7th day of the experiment. The findings of Joshi and Misra (1974); Prasad (1977) and Sethuraman (1976) agree with the present observations. Decrease in total volatile fatty acid production may therefore be attributed to high rumen pH, decreased microbial population in rumen leading to decrease in digestibility and fermentation (Mehrez et al., 1977) during ruminal stasis.

Nonsignificant change was noticed in concentration of both sodium and potassium in rumen liquor. Hyponatraemia due to increased urinary excretion of sodium in experimentally induced acidosis in sheep has been reported (Nakota et al., 1977). The present observations are in accordance with findings of Cakala et al. (1980) in bovines.

A significant ($P < 0.01$) decrease in calcium concentration of rumen liquor was observed in the present study. Wheat straw has been reported to be poor in calcium

content (Malik, 1984). Oxalates present in straw chelate the calcium content making it unavailable and in addition alkaline pH also hampered the absorption of calcium from rumen (Blood et al., 1983). Slight decrease in both magnesium and inorganic phosphorus levels of rumen liquor was observed in the present study. Low intake of these ingredients, as wheat straw being poor in respect of magnesium and phosphorus (Malik, 1984) was responsible for low levels.

A significant decrease in thiamine concentration of rumen liquor was in accordance with findings of Hoflund (1967); Gupta et al. (1976) and Blood et al. (1983). Marked reduction in rumen microflora (Bachdel and Honeywell, 1927 and Bachdel et al., 1928) and liberation of thiaminase type I enzyme by certain rumen bacteria (Morgan and Lawson, 1974) were the probable reasons for the decreased level of thiamine in rumen liquor.

The serum ammonia nitrogen concentration did not vary much. It ranged between 0.45 ± 0.038 to 0.51 ± 0.013 mg% throughout the experiment. Signs of ammonia toxicity in cattle was reported to occur when blood ammonia nitrogen exceeded 0.7 mg% (Webb et al., 1972). In the present experiment serum ammonia nitrogen concentration was much lower.

The concentration of sodium and potassium in the serum fluctuated marginally similar to that of rumen liquor. However no correlation between rumen and serum sodium and

potassium concentration has been reported (Telle and Preston, 1971). Increased sodium excretion through kidneys in lieu of H^+ ion in metabolic alkalosis in cattle has been reported (Kuiper, 1980) which might have caused slight decrease in sodium level in the present experiment.

A significant decrease in serum calcium from 10.62 ± 0.180 to 9.11 ± 0.371 was recorded on 11th day in the present study. The hypocalcaemia can be attributed to low dietary intake of calcium through wheat straw (Malik, 1984) and decreased absorption from intestine in alkalosis as reported by Sethuraman and Rathor (1979) and Dave (1980) in clinical cases of alkaline indigestion. A positive linear relationship between rate and amplitude of rumen contractions and plasma calcium in both sheep and cattle has been reported by Daniel (1983).

Serum magnesium and phosphorus concentration decreased nonsignificantly following exclusive feeding of wheat straw. Wheat straw has been reported to be rich in fibre and lignin content while poor in respect of calcium, magnesium and phosphorus. Due to high lignification, the digestibility of most of the nutrients has been reported to be low (Malik, 1984). Also elevated ammonia nitrogen and decreased TVFA concentration in rumen liquor hinder the absorption of magnesium from rumen (Horn and Smith, 1976 and Regier, 1980). A negative linear relationship

between magnesium absorption and ammonia nitrogen concentration while a positive relationship between magnesium absorption and volatile fatty acid concentration in the rumen of sheep has been reported (Martens, 1980) which support the present finding.

A significant decrease in thiamine concentration in blood was recorded in the present investigation which is in agreement with findings of Gupta et al. (1976) and Sinha (1981). Low thiamine level in rumen liquor lead to hypothiaminosis and provoked the clinical manifestation like anorexia, emaciation, muscular weakness and progressive recumbency.

The physical nature of urine samples did not vary much during the experimental period. Qualitatively glucose, albumin, ketone bodies, bile salts were not detected. These findings are in agreement with results of Sethuraman (1976) and Dave (1980). However they reported presence of albumin in traces in experimentally induced acute alkalosis.

Out of five, three calves died on 11th, 15th and 17th day of the experiment. The death was owing to acute impaction of rumen, inanition and progressive dehydration. Crossly marked congestion and submucosal/patches in intestinal mucosa were observed.

Early adoption of treatment has been recommended for rapid recovery in experimental as well as clinical cases of alkaline indigestion and impaction in bovines

(Misra and Singh, 1974; Bartley et al., 1976; Davidovich et al., 1977 and Sethuraman and Rathor, 1979).

On the basis of clinico-biochemical alterations in animals of group II, therapeutic measures were adopted in animals of group III on 11th day after induction of ruminal atony by feeding wheat bhusa exclusively. The aim of parenteral administration of calcium, magnesium and thiamine, after neutralisation of pH of rumen was to supplement the calves with these ingredients. Four out of five calves were cured successfully. Signs of therapeutic response were observed on 13th day in all the animals. All the animals regained normal rumen motility and appetite by 17th day of experiment.

Following therapy on 11th day, the colour, consistency and odour of rumen liquor became normal on 14th day of observation. The SAT though delayed upto 14th day, came near base value on 15th day of observation. The motility of protozoa remained sluggish initially even after treatment and became moderate on 13th day. From 15th day onwards rumen protozoa had attained normal vigorous motility. The concentration of protozoa regained to normal by 17th day.

The pH of rumen fluid which had increased to 8.14 ± 0.064 on 11th day decreased to 6.88 ± 0.105 on 14th day due to neutralisation with weak acetic acid. However, the return of pH to normal range was gradual.

The total protozoal count, which was significantly low on 11th day increased gradually following treatment. However, it was significantly ($P < 0.05$) low even on 15th day. This could be due to slow and gradual decrease in ruminal pH. Sethuraman and Rathor (1979); Karunanidhi (1983) and Blood *et al.* (1983) have advocated rumen cud transplantation for immediate reestablishment of rumen protozoa. There was gradual increase in differential count of Holotrichs i.e. Isotricha and Dasytricha species during post treatment period and even on 17th day of observation their percentages were lower than zero day values. Entodinium showed decreasing trend after treatment. Epidinium and Diplodinium had fluctuating pattern during post-treatment period. The unidentified and other protozoa showed decreasing percentage following treatment. There was gradual and slow reestablishment of rumen microbial population, which is in confirmity with findings of Prasad and Rekib (1975) and Dave (1980). The predominance of Gram negative bacteria comprising of rods, coccobaccilli and cocci was noticed upto 15th day of observation. Thereafter some Gram +ve bacteria were also detected in stained rumen fluid smears.

Rumen fluid ammonia nitrogen level, which increased to 22.95 ± 0.658 mg% on 11th day declined to 13.15 ± 0.517 mg% on 15th day. The improvement of reticulo-ruminal motility, pH and microbial population lead to decreased formation and/or

increased utilization of ammonia nitrogen in the rumen by the microbes. TVFA concentration of rumen fluid which decline to 36.54 ± 1.298 mEq/L on 11th day showed a gradual, but significant increase by 17th day, following therapy. The regain in TVFA concentration might be due to establishment of normal rumen microbial fermentation. This is in accordance with findings of Karunanidhi (1982).

Though the concentration of sodium and potassium changed partially, the level came to normal following treatment. Calcium level of rumen liquor, which had decreased significantly, showed increasing trend after treatment but was low even on 17th day as compared to zero day value. The increase could be attributed to normalisation of rumen environment and consumption of green fodder and parenteral administration of calcium borogluconate (Thiocal). Magnesium and phosphorus content of rumen fluid also increased after commencement of therapy.

Thiamine concentration of rumen liquor improved gradually. Even on 17th day thiamine concentration was significantly ($P < 0.05$) low as compared to zero day value. This gradual increase in ruminal thiamine might be due to slow establishment of normal rumen flora (Karunanidhi, 1982).

Serum ammonia nitrogen decreased gradually following therapy. Parenteral fluid therapy helped in normalisation of both sodium and potassium levels in serum.

Serum calcium concentration regained its normal level after treatment and reached near zero day value on 15th day of observation. Parenteral administration of Thiactal could have helped to restore the serum calcium level. Magnesium and inorganic phosphorus concentration in serum followed the trend of calcium and reached normal values on 17th day.

Following therapy, the blood thiamine level increased markedly as thiamine hydrochloride was included in the therapy. Regain of microbial synthesis of thiamine in the rumen might have added to restore normal level in blood.

SUMMARY AND CONCLUSION

Rumen motility is an index for determining the digestive function in ruminants, while rumen microbes play a major role in the digestion of ingested feeds and fodders. Exclusive feeding of poor quality roughages to cattle during the period of non-availability of greens and pasture grasses upsets the ruminal fauna leading to ruminal atony. The disturbance is clinically characterised by inappetence leading to dullness, gradual loss of condition, rough hair coat, drop in milk yield in lactating and poor working capacity in drought animals. In the present project efforts have been made to elucidate the effects of exclusive feeding of wheat straw on the rumen as well as its microbes and allied clinico-biochemical aspects in cattle.

Fifteen crossbred calves of 1-1½ years age were utilised in the present study. Before the start of the experiment, animals were kept under observation for a period of one month. The animals were then fitted with rumen fistula, and were randomly divided into three groups. Animals of group I were kept as healthy control. For establishing the normal values, rumen fluid, blood and urine samples were collected at 48 hours interval from the animals of group I. Physical characteristics, microbial population and biochemical constituents of rumen liquor like ammonia nitrogen, total volatile fatty acids, calcium, magnesium, phosphorus, sodium, potassium and thiamine were

estimated. Blood constituents like ammonia nitrogen, calcium, magnesium, phosphorus, sodium, potassium and thiamine were determined. Qualitatively urine samples were also examined.

In the animals of group II, ruminal atony was induced experimentally by intraruminal administration of wheat straw @ 2 kg per 50 kg body weight daily in addition to their normal intake. Ruminal stasis was observed between 9-11th day of the experiment. Clinical manifestations were recorded daily while collection of rumen fluid, blood and urine samples was done on every alternate day. The clinical signs exhibited by the animals were progressive inappetence, dullness and depression, loss of condition, severe grinding of teeth and passage of constipated faeces. The rumen was atonic, doughy and impacted. Out of five three calves died on 11th, 15th and 17th day of the experiment.

The colour of rumen fluid changed to yellowish and watery in consistency with offensive smell. Both motility and concentration of rumen protozoa diminished significantly. Sedimentation activity time was delayed and even absent. Total protozoal count as well as differential count of Holotrichs decreased significantly. Gram stained rumen fluid smears revealed predominance of Gram negative bacteria.

Biochemically there was significant increase in pH of rumen fluid and ammonia nitrogen concentration while

significant decrease in the concentration of total volatile fatty acids, calcium and thiamine in rumen liquor. Significant alterations were also recorded in serum calcium and thiamine concentration. No significant alterations were noted in level of electrolytes either in the rumen liquor or in the serum. However magnesium and phosphorus concentrations showed a gradual decrease both in the rumen liquor and serum. Qualitatively urine samples were negative for albumin, glucose, ketone bodies and bile salts.

In the animals of group III, ruminal atony was induced experimentally as in the animals of group II and based on the finding in animals of group II, the therapy was undertaken soon after the development of ruminal atony. The treatment comprised of administration of weak acetic acid with bolus Bovirum (Sarabhai chemicals) and powder Digestovet (Vets Pharma Pvt.Ltd.) intraruminally along with parenteral administration of Thiactal (Wockhardt) and Norwal^m saline (Luna Pharma).

Following treatment for three days there was gradual decrease in rumen pH and increase in total protozoal count. Clinically the appetite restored by 14th day. Most of the biochemical alteration in the rumen liquor regained approximately to normal levels by 17th day, following treatment. However ruminal thiamine content did not improve to the desired extent. The biochemical alterations

in blood also improved by 17th day. All the animals responded well to the treatment except one which died on 11th day of the experiment.

Based on the above observations, it is concluded that

- (i) Exclusive feeding of wheat straw to the cattle leads to significant alterations in the microbial population, cessation of ruminal motility leading to digestive disorder which is clinically characterised by progressive inappetence, weakness, emaciation and even death of the animals.
- (ii) Clinical examination of rumen and physical as well as biochemical evaluation of rumen liquor are essential for judging the degree of dysfunction due to exclusive intake of wheat straw in cattle.
- (iii) Neutralisation of ruminal pH with weak acetic acid and oral supplementation of Bovirum (Rumenotonic) and Digestovet (Stomachic), along with parenteral administration of Thiactal and Normal saline proved effective in curing the condition.

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