STUDIES ON THE DIAGNOSIS AND TREATMENT OF ANOREXIA IN MILCH BUFFALOES

A Thesis submitted to the
KONKAN KRISHI VIDYAPEETH, DAPOLI
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Master of Veterinary Science

in

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by

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C E R T I F I C A T E.

Certified that the thesis entitled " STUDIES ON THE DIAGNOSIS AND TREATMENT OF ANOREXIA IN MILCH BUFFALOES " submitted by RAJIV VASANTRAO GAIKWAD in partial fulfilment for the degree of MASTER OF VETERINARY SCIENCE (M.V.Sc.) in MEDICINE, embodies the results of the record of bonafide research work carried out by him, under the guidance of University Teacher, Dr. S. JAGADISH, and the same is to our satisfaction.

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CONTENTS.

Introduction

INTRODUCTION

Buffalo is the main milch animal species in our country. Contribution of buffaloes in the total milk production is larger than that of the cattle. India posseses 34 million milch buffaloes (Tata *et al* 1990).

Indigestion is a complex subject with many types, manifestations and variations and is one of the many conditions confronting the veterinarian in which a good correlation of history, clinical examination and observation has to be done. Anorexia directly reflects on the milk yield of the dairy animals and thus on the economy of the farmer and the nation. The production losses caused by diseases varies between 30-40% of the gross livestock production. A report from Madras indicated the hospital incidence of Bovine indigestion of about 53% during the period from 1963-1968 (Balasubramanian, 1970), therefore the resulting economic losses can be enormous. Complete anorexia persisting for more than 3-5 days is considered unfavourable and persistent inappetence suggests a chronic lesion usually with an unfavourable prognosis (Blood and Radostits, 1989). It is therefore logical to check the condition of the contents of the rumen at an early stage in such cases so as to take prompt remedial measures and thereby prevent economic losses.

The microflora of ruminants can digest cellulose, ferment carbohydrates to volatile fatty acids and convert nitrogenous substances to ammonia, amino acids and proteins. In certain circumstances the activity of the flora can be suppressed to the point that digestion becomes abnormal or ceases. Incorrect diet, prolonged starvation or inappetence and hyperacidity which occurs in engorgement on grain will impair microbial digestion. Oral administration of drugs (antimicrobials) has a deliterious effect on bacteria, yeasts and protozoa; it might even alter drastically the pH of the rumen contents (Fraser *et al* , 1986).

Simple correction of ruminal pH does not revive the appetite immediately in all cases (Prasad *et al* 1973). Various products like probiotic (Bioboost), ruminotoric (Floratone) and herbal preparation (Rumbion) are now available for field use. However efficacy of these products has not been completely evaluated in clinical cases of anorexia in dairy animals. Therefore the present study was undertaken with the following objectives in view :

1) To diagnose the cases of anorexia.
2) To study the changes in the rumen liquor before and after treatement in cases of anorexia.
3) To study the biochemical changes as a result of anorexia.

4) To study the therapeutic efficacy of preparations like probiotic (Bioboost), rumenotoric (Floratone) and herbal preparation (Rumbion) in clinical cases of anorexia.

Review Of Literature

REVIEW OF LITERATURE.

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Anorexia is a symptom of many diseases or conditions and not a separate disease entity. There are several reports on the different aspects of anorexia syndrome. Therefore the pertinent literature has been reviewed under the following headings.

2.1 Classification

2.2 Diagnosis

2.3 Changes in rumen liquor

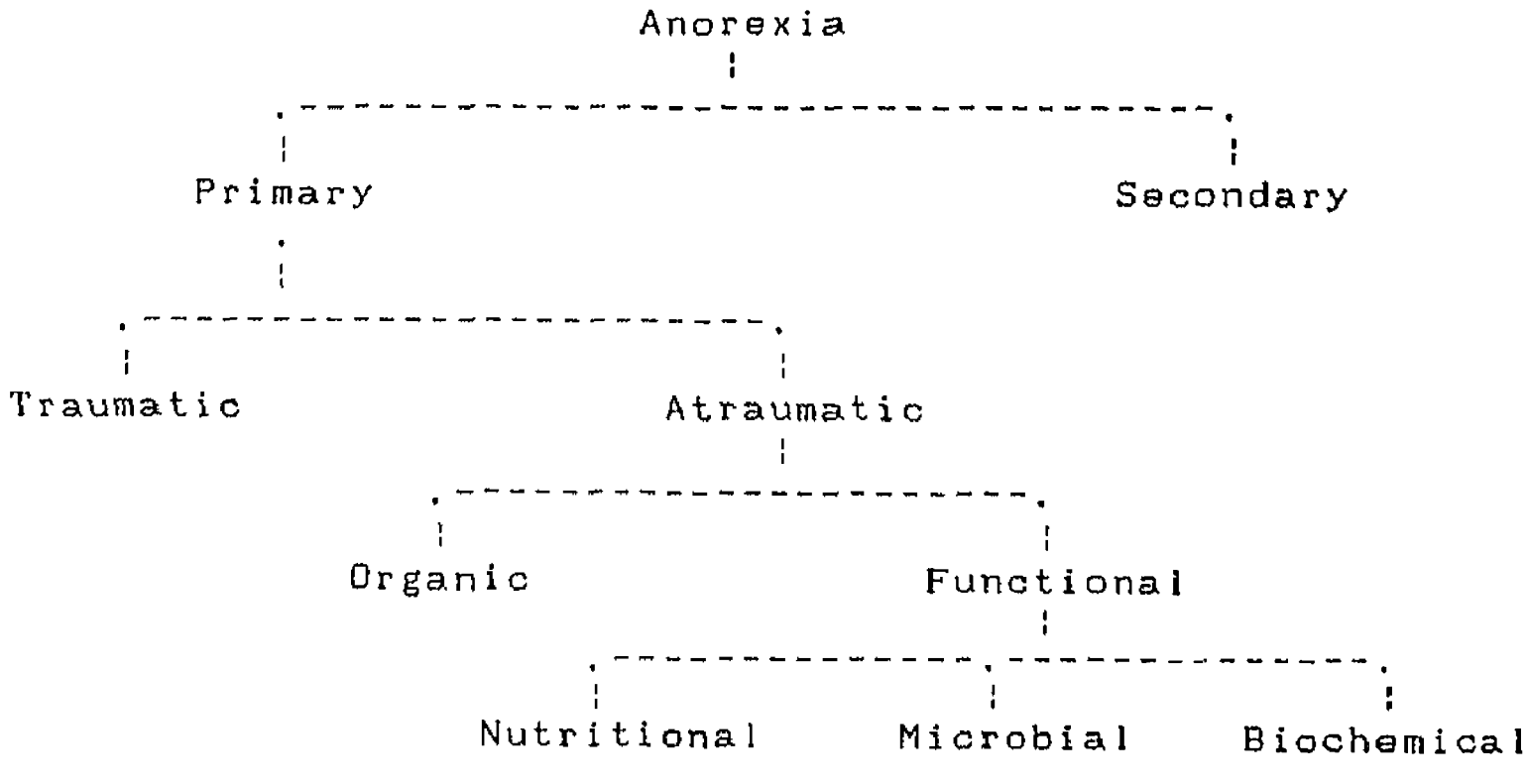
2.4 Haematological and biochemical changes

2.5 Treatment

2.1 CLASSIFICATION.

Hoflund (1967) classified rumen digestive disorders into three types, based on the reactions of the ruminal pH as acid indigestion (pH 4.0- 5.5), alkaline indigestion (pH 7.5- 8.5) and indigestion with normal pH (pH 6.0-7.0).

Kadvekar and Murkibhavi (1971) classified anorexia that was commonly encountered in ruminal and reticular ailments as follows.



Dirksen (1983) denoted the term " indigestion " to the diseases of the reticulum and rumen. The first differentiation is made between primary and secondary indigestions. While in the former the rumen and reticulum are primarily affected, the latter form of indigestion occurs secondary to diseases of other organs or as accompanying symptoms associated with severe general diseases.

He classified the indigestion of the forestomach as under :

* Primary indigestions (Idiopathic disorders of the rumen and reticulum).

Motor dysfunctions -

Diseases of the reticulo-ruminal wall.

Disorders of nervous regulations of forestomach motility.

Mechanical obstruction to passage of ingesta.

Disorders of microbial-biochemical digestion.

- accompanied by high pH of rumen fluid.
- accompanied by low pH of rumen fluid.
- accompanied by normal or lowered pH of rumen fluid.

* Secondary indigestions (rumen and reticulum secondarily involved).

- Secondary motor insufficiency of rumen and reticulum.
- Secondary simple insufficiency of the ruminal flora and fauna.
- Latent hyperacidity of reticulo-ruminal ingesta due to abomasal reflux.

2.2 DIAGNOSIS.

2.2.1 *History.*

Dirksen (1983) suggested that the history with a view to the ration is important in all cases of indigestions and will help in the assessment of biochemical changes taking place in the rumen contents during microbial digestion.

2.2.2 *Rumen Motility*

Boddie (1946) opined that interference with reticulo-ruminal activity was considered one of the earliest signs of digestive disorders and hence the motility of the rumen could be taken as a clinical index for diagnosis of rumen disorders.

Hoflund (1967) reported that reduction in reticulo - ruminal motility in acid and alkaline indigestion may be related to acute pH variation.

Prasad (1979) indicated that there was no definite relationship between rumen pH and motility of reticulo-ruminal musculature in clinical acidosis and alkalosis.

Blood *et al* (1983) found that bacterial digestion and fermentation and physical maceration by contraction of the stomach walls are the two main functions of the forestomach and the two are interdependant. Thus abnormality of one leads to the abnormality of the other and of the two, the motility is most readily examinable. Ruminal motility is therefore used as an index of digestive functions in the ruminant.

Dirksen (1983) outlined the procedures for diagnosing the diseases of the forestomach as under :

1) Establishing the history with special consideration of the feeding practice.
2) Thorough clinical general examination.

3) Specific examination of the forestomach system including
   - examinations of both the flanks, curvature of the left flank, rumen motility, outlines of the abdominal wall, palpation, auscultation, percussion, pain sensitivity tests etc.
4) Clinical evaluation of the rumen fluid for colour, odour, viscosity, sedimentation activity test (SAT) or flotation time, pH, determination of methylene blue reduction time (MBRT), examination for protozoa and bacteria.

Arle (1989) observed decreased rumen motility in anorectic cows (0 to 2/3 min) as compared to the control group (1 to 3/ min).

2.2.3 RUMEN FLUID

2.2.3.1 *Sampling Technique*

Gall *et al* (1949) used a large-bore rumen tube (3/4") for withdrawing the sample from cattle and medium-bore rumen tube (1/2") for sheep. After the tube had been inserted into the rumen, it was attached by means of an adapter in rubber stopper to a suction flask by a rubber tube and about 200-500 ml of the rumen contents were drawn out by suction.

Pounden (1954) used stomach tube made up of rubber in various sizes to collect rumen samples for examination and in larger quantities for inoculation and transfusion. He preferred

stiff rubber tubing with 1/2" in outside diameter and three feet in length for small calves.

For large animals, tubes varying in size upto the large colorado tube was recommended. He stated that after proper location of the tube in the rumen, a back-and-forth motion of few inches to a foot caused a gurgling sound as the tube splashed in the liquid. The same motion encouraged ruminal contractions and flow of liquid portion of the contents out through the tube. He further indicated that when the liquid quantity was low, some assistance could be obtained by upward pressure in the lower left abdominal region.

Raun and Burroughs (1962) used a specially designed suction strainer made up of stainless steel, attached to the suction line of three feet length and 1/4" outside diameter to which a 50 ml hypodermic syringe was connected. The suction line was passed inside a 3/4 x 18" rubber guide tube and used for ruminal fluid collection.

Radostits and Magnusson (1971) used a Kingman stomach tube with an outside diameter of 1 1/2" and inside diameter of 1" and 10 feet long for emptying the rumen of cattle affected with digestive dysfunctions. Rumen was emptied by allowing rumen contents to flow out by gravity.

Prasad (1976) used a 4" long aspiration needle fitted with a leurlock-mount syringe and collected rumen fluid by puncturing the abdominal wall at left paralumbar fossa for various biochemical studies in clinical cases of bloat.

Gnanaprakasam (1979) used mouth gag and rumen fluid extraction pump named as ' Thaman's Rumen Fluid Extraction Pump' consisting of four parts; a suction strainer, a four meter long nylon tube, a suction apparatus and a sampling bottle.

Dirksen (1983) used suction pump attached to the flexible stomach tube and sampling bottle. He suggested that the stomach tube should be of sufficient length (about 2.30 meters) and the head of the flexible stomach tube should become bent so as to be able to obtain rumen fluid from the area of the ventral ruminal sac.

Fouad and Ibrahim (1988) removed reticular, abomasal and duodenal fluid samples from six buffaloes with a simple device formed of specially designed polyvinyl plastic tube and a double channel suction pump of 50 ml capacity. The tube was introduced through the rumen cannula and passed through the rumino-reticular orifice, reticulo-omasal orifice and upto the duodenum through the pyloric orifice.

Arle (1989) used rumen fluid extraction pump as a satisfactory method for collecting the rumen fluid samples for diagnosis as well as therapeutic purpose.

2.3 CHANGES IN RUMEN LIQUOR

2.3.1 *Colour* :

Dash and Misra (1972) observed yellowish brown colour of rumen fluid in cattle suffering from primary indigestion due to a sudden change in feed.

Dirksen (1983) recorded that the colour of the rumen was milky-grey in acidosis, greenish-black in prolonged stasis and putrefaction of reticulo-ruminal contents, whereas dark brown or green in simple inactivity of flora and fauna.

Blood and Radostits (1989) mentioned that the colour, depending on the feed to a limited extent, will be green, olive green or brownish green. At pasture, the colour is very green, with root crop the colour tends to be grey and with silage or straw the colour is mostly of a yellow brown nature. The colour of the rumen contents will be milky grey in grain overload, and greenish black in cases of stasis of long duration and where putrefaction is occuring within the rumen.

2.3.2 *Odour* :

Dirksen (1983) noted that odour of the rumen contents was rotty/foul in protein putrefaction, while intensely sour odour was noted in excess of lactic acid formation due to carbohydrate engorgement.

Blood and Radostits (1989) stated that the odour of the rumen contents is normally aromatic and, although somewhat pungent, not objectionable to the nose. A mouldy, rotting odour usually, indicates protein putrefaction and an intensely sour odour indicates an excess of lactic acid formation, due to grain or carbohydrate engorgement.

Garry (1990) recorded pungent, sour or acidic odour in ruminal acidosis cases; Foul and putrid odour in the cases of ruminal putrefaction with protein decomposition and ammoniacal odour in the cases of urea poisoning.

2.3.3 *pH*

Nichols (1957) suggested that practical determination of pH of rumen contents of sick animals will allow better selection of therapy.

Allison *et al* (1964), Ahrens (1967) and Joshi (1969) observed that abnormally high or low pH of rumen fluid was associated with anorexia, decreased motility and changes in microbial population.

Pant and Roy (1970) reported that pH of the rumen fluid was a reliable criterion for measuring the microbial fermentation rate.

Swenson (1977) indicated that the pH of rumen fluid did not deviate far from neutrality and it was usually within the range of 5.8-7.0. There was a decrease in pH after feeding, the speed and extent of the decrease being related to the nature of diet.

Hedaoo *et al* (1982) studied the indigestion in the buffaloes and noted that the pH of the ruminal fluid ranged from 3.00-5.5 in acid indigestion, 7.9-9.5 in alkaline indigestion and 6.5- 7.0 in non specific anorexia.

Dirksen (1983) studied putrefaction of the rumen content, which was black green in colour, foul odour and pH range of 7.5 to 8.5. Further he noted that there was great reduction in the number of protozoa.

Sopori and Prasad (1987), experimentally induced ruminal atony in five male cross-bred calves of 1 to 1 1/2 year age, engorged with 2 kg/ 50 kg body weight wheat straw. They found increase in rumen pH upto a maximum of 8.14 on 11th day of post engorgement.

Garry (1990) found that, the lower end of the pH range indicates rapid fermentation of readily digestible carbohydrates (Concentrate and young grass). The pH rises

when the fermentation slows. Ruminal fluid with a high pH results from the slow fermentation of feeds with a high fiber contents.

2.3.4 *Sedimentation activity test (SAT)/Flotation time.*

Nichols and Penn (1958) opined that cellulose digestion test and SAT were the simple diagnostic tests used to detect unfavourable changes in ruminal ingesta.
Misra and Tripathy (1963) observed a significant rise in SAT when the animals are fed exclusively on paddy straw.

Randhawa *et al* (1989) opined that complete absence of SAT had resulted from destruction of the normal rumen microflora and shift in their pattern from predominantly Gram negative to Gram positive.

Arle (1989) found increased SAT (9 to 15 min) in acid indigestion, alkaline indigestion and in non-specific anorexia cases of cattle.

Garry (1990) opined that watery samples with inactive microflora show rapid sedimentation and little floatation. This type of fluid is seen with ruminal acidosis and prolonged anorexia and when the microflora are inactive because the animal is being fed undigestible roughage. Frothy bloat and some cases of vagal indigestion, may show no appreciable sedimentation or floatation. An active

microfloral population is necessary to produce gas that helps fibrous material to float.

2.3.5 *Methylene blue reduction time (MBRT)*

MBRT reflects the anaerobic fermentation metabolism of the bacterial population. Reduction of the dye is a consequence of the redox potential of the fluid.

Dirksen (1983) suggested the use of MBRT to assess the microbial status of ruminal fluid. The test was performed by adding 20 ml of rumen fluid at body temperature to one ml of 0.03 % methylene blue solution and subsequently measuring the time needed to decolourise the fluid. He opined that reduction time of less than 3 minutes, indicated highly active ruminal microflora, whereas a marked increase in MBRT with a drop in pH below 5.0 was noted in acidosis. Reduction time in moderately active ruminal flora was 6 minutes; longer reduction time is indicative of decreased activity or acidosis.

Arle (1989) observed increased MBRT in anorectic cows (9 to 14 min) compared to control group (2 to 4 min).

Garry (1990) used 0.5ml of 0.3% of methylene blue solution, in which 10 ml of ruminal content was added and the reduction time was noted. He found that ruminal fluid from grain-fed cattle tends to reduce the dye quickly (in 3 or 4 minutes), while fluid from cattle fed mostly on hay rations tends to

take longer (5 or 6 minutes). With inactive flora, the reduction time may be prolonged to 10 minutes or more.

2.3.6 *Total protozoal count.*

Rumen protozoal population was considered as an index of good health of the individual animal. Hungate (1966) noted that total count varied from none to as high as 5×10^6 and usually within the range of 2×10^5 to 2×10^6 per ml of rumen fluid.

Hungate (1966) and Dirksen (1979) reported that, the examination of ruminal protozoa could be extremely helpful in preliminary diagnosis of alimenatary tract dysfunction.

Pant and Roy (1970) found that the mean protozoal concentration in rumen liquor of buffalo was $21.29 \pm 0.629 \times 10^4$ and in zebu it was $21.66 \pm 0.693 \times 10^4$. They also noted that the lowest counts were obtained 5 hours after feeding. Total protozoal number has been reported to bear an inverse relationship with the acidity of rumen fluid.

In a clinical study on seven cattle suffering from acute indigestion, Dash *et al* (1972) evaluated the rumen fluid for total protozoal count. They could not observe any living protozoa in rumen fluid having a pH lower than 5.5.

Dirksen (1983) noted that the large species of the protozoa are the first to vanish with disorders of the ruminal

digestion, the medium sized are next, and small protozoa disappear last.

Arora (1983) stated that the number of protozoa varies greatly in rumen with the type of diet, age and breed of the animals. It may exceed 1×10^6 per gram of rumen content and their total mass is roughly equal to bacteria.

The ciliate protozoa are strictly anaerobic, when oxygen content or pH of the ruminal contents is high, they cannot form cysts to resist adverse environment and are thus rapidly killed.

Randhawa *et al* (1989) studied the effect of lactic acidosis on microbial changes in rumen fluid of buffalo calves. In their studies on eleven, one year old male murrah buffalo calves, engorged with wheat straw, they noticed a significant decrease in average total protozoal count by six hours of the induction of acidosis and complete absence of protozoa at subsequent intervals. But in subacute acidosis, reappearance of protozoa was observed by 120 hours. The total protozoal count was found to be $0.59 - 0.04 \times 10^5$ per ml of rumen fluid at 168 hours.

Arle (1989) noted decreased total protozoal count in anorectic cows (0.25 to 1.75×10^5/ml of rumen fluid).

Blood and Radostits (1989) stated that microscopic examination of few drops of rumen fluid on a glass slide with a low power field will reveal the level of protozoal activity. Normally five to seven protozoa are active per low power field. In lactic acidosis the protozoa are usually absent or a few dead ones are visible.

Prasad and Pradhan (1990) found that, rumen protozoal counts were higher in buffaloes than in cattle irrespective of level of the concentrates in diet based on wheat straw. Average total protozoa at 0 hour in buffalo was $4.8 \pm 1.5 \times 10^5$ per ml and in cattle $2.8 \pm 1.3 \times 10^5$ per ml and at 4 hour after feeding it was $9.2 \pm 4.3 \times 10^5$ per ml in buffalo and $3.1 \pm 0.7 \times 10^5$ per ml in cattle.

Garry (1990) opined that the observed protozoa can be grouped either by size (small, medium or large) or by morphology (holotrichous, with cilia over the entire body, or oligotrichous, with cilia only near the mouth) An absence of motility may indicate chilling, if the sample has not been kept warm. Protozoal numbers and motility will decline greatly if the ruminal pH drops sharply or if other adverse fermentative conditions are present. They are good indicators of the rumen condition.

2.3.7 *Iodophilic activity of protozoa.*

Pant (1966) stated that the iodophilic micro-organisms play an important role in the degradation of starch, cellulose and

other carbohydrate constituents of the animal's ration. Further, he added that a consistant and significantly higher number of an iodophilic micro-organism in buffalo rumen contents has always been observed in comparison to zebu cattle. Iodophils act as better protein synthesizers and, therefore are better converter of ingested feed.

Pant and Roy (1970) stated that the iodophilic organisms are protein synthesizers. Thus higher number of iodophils in buffalo suggest the possibility of greater protein synthesis in their rumen.

Misra *et al* (1972) used Lugol's iodine to trace stored starch inside the holotrichids which gave blue colour, whereas the oligotrichids showed brown colouration due to their glycogen deposits.

2.3.8 *Proportion of dead to live protozoa*

Weir (1967) stated that trypan blue is the dye most commonly used for reading cytotoxic tests. Trypan blue is made up as a 1% stock solution and is diluted 8 - 10 times with isotonic saline.

Jagadish (1977) used 0.1% trypan blue solution in " trypan blue exclusion technique", for viable count. 0.1 ml of cell suspension was diluted with 0.9 ml of 0.1% trypan blue solution and a haemocytometer was charged with a drop of this

mixture. After allowing to settle, the unstained cells (viable cells do not take up the stain) were counted in four large squares just as for WBC counting. The average number of viable cells of the four square multiplied by 10^6 gave the total number per ml of the suspension.

2.3.10 *Total bacteria.*

Gall *et al* (1947) stated that the direct bacterial counting technique was found to be most successful. It involved the use of nigrosine to colour the background, leaving the bacteria unstained.

Gall *et al* (1949) used smears of 1:10 dilution of rumen contents, stained with Grams stain and counted for bacteria of cattle and sheep. They observed, averages of about 50 billion bacteria per gram of fresh rumen content on winter ration. Both species gave higher slide count, when on pasture, the average being 96.1 billion and 85.4 billion respectively.

Pant and Roy (1970) found that the bacterial concentration may be as high as 21×10^9 per ml of rumen liquor in zebu and approximately 25×10^9 per ml of rumen liquor in buffaloes.

2.4 HAEMATOLOGICAL AND BIOCHEMICAL STUDIES.

Dash *et al* (1972) found an increase in haemoglobin levels with simultaneous rise in total RBC count and PCV in acute

indigestion. Further, they also observed leukocytosis with neutrophilia and lymphopenia. They opined that the haemoconcentration might be due to dehydration.

Misra and Singh (1974) found haemoglobin values to be on an average 11.2 gm % in anorexia due to acid and alkaline indigestion in cattle, which was similar to the value for healthy cattle in their studies. There was slight increase in leukocyte count, but PCV, lymphocyte and eosinophil percentage was slightly less in anorectic animals.

Joshi and Misra (1976) observed increased SGOT activity in 24.3% out of 37 specimens examined in buffaloes suffering from ruminal disorders. The SGOT activity of 119 units per ml was noted with abnormally high ruminal pH 8.5 and low activity of 10.0 units per ml in animals with fairly normal ruminal pH, but there was no correlation between ruminal pH and enzyme activity in blood.

Sethuraman and Rathor (1979) recorded increase in blood glucose and serum transaminases (SGOT and SGPT) in acid and alkaline indigestion.

Hedaoo *et al* (1982) recorded observations on 56 cases of buffaloes suffering from indigestion and found an increase in haemoglobin percentage, slight increase in neutrophils and reduction in lymphocytes in all types of indigestions. There was also an increase in the transaminase activity.

Cornelius and Kaneko (1983) reported that SGOT is not liver specific in ruminants, yet the rise might be either due to affections of hepatic parenchyma or due to absorption of toxic metabolites from the rumen, produced by bacterial degradation of proteins and putrefaction resulting into the leakage of large amounts of SGOT from hepatic cells into the blood.

Sastry (1983) reported that neutrophilia in conditions where there is release of corticosteroid as in states of stress - pain, anaesthesia, trauma, manipulation in surgery, neoplasia etc.

Ahuja *et al* (1988) reported hyperglycaemia, elevated SGOT levels in urea-induced ammonia toxicity in buffalo calves.

Galhotra and Gupta (1988) reported increase blood cholesterol level in experimentally induced aflatoxicosis in buffalo calves which was attributed to its decrease uptake by the damaged liver where cholesterol is metabolised to bile acids.

Singh and Kasarlikar (1988) reported hypoglycaemia and incresed serum cholesterol levels in clinically ketotic buffaloes.

Arle (1989) reported significant increase in blood glucose, SGOT levels and neutrophil count and decrease in lymphocyte count in cows suffering from primary anorexia.

Singh *et al* (1989) reported high levels of blood glucose in buffaloes suffering from indigestion as compared to normal ones. The hyperglycaemia could be attributed to the stress of digestive disorder leading to release of adrenocorticoids which cause glycogenolysis and there by hyperglycaemia.

Patel *et al* (1990) recorded averages of total cholesterol (93.79 mg per 100 ml), free cholesterol (13.81 mg per 100 ml) and ester cholesterol (79.98 mg per 100 ml) in surti buffaloes and found that these values were lower than that of cattle. They further observed that, the levels of total cholesterol and ester cholesterol were compartively higher at a phase close to ovulation and follicular phase.

2.5 TREATMENT

2.5.1 *PROBIOTIC*

Probiotic is the term coined by Parker (1974) to describe organisms and substances which contribute to intestinal microbial balance. It originated from two greek words meaning " for life " and contrasted with the term antibiotic which means " against life ".

Belief in the beneficial effects of probiotic comes from Metchnikoff (1908), who contended that the longevity of the Bulgarian peasants was related to their consumption of large amounts of milk fermented with organisms such as *Lactobacillus acidophilus*. He explained that the detrimental

microbes in the intestinal tract excreted a substance that was harmful to the host. Through the constant infusion of the " friendly " organisms in the diet, colonisation of the gastro-intestinal tract by disease-causing "unfriendly" organisms were prevented and there by health and life expectancy was improved. Thus the concept of microbial inoculation based on the principle of "competitive exclusion" was established.

Walassova and Nomotelnow (1933) isolated *Lactobacillus sporogenes*, a probiotic species of *Bacillus coagulans*. As the name indicates *Lactobacillus sporogenes* is a lactic acid producing and spore forming bacillus. They are Gram positive, aerobic and microaerophilic and thermophilic in nature.

Probiotics are bacterial cultures or preparations that favour or promote life. They are administered as feed additives. Lactobacillus forms a more common group amongst probiotics. These organisms are mainly responsible for fermentation .

Tagg *et al* (1976) found that many of the lactic acid bacteria posses an ability to produce bacteriocins which are antibiotic-like compounds active against closely related bacteria.

Fuller (1977) adjusted the pH of agar medium to 4.5 with lactic acid or hydrochloric acid and observed that the growth of *E.coli* was inhibited by low pH.

Gilland (1979) confirmed that, most of Gram positive-lactic acid bacteria (Lactobacilli and Streptococci) are resistant to lysozyme than other bacteria.

Gilland (1979) and Conway *et.al.* (1987) found that the Lactobacilli are located in the intestines and are resistant to the levels of acidity encountered in the stomach.

Savage (1981) opined that a strain of certain endogenous Latobacillus species is known to associate with epithelial surfaces in the alimentary canal of some mammals and birds. The bacterial strains colonize the surface of the alimentary canal early in animal's life and adhere to it. The mechanisms are relatively specific for the animals depending upon the species from which the strains are derived.

Kim and Gilland (1983) found that *Lactobacillus acidophilus* is able to survive and grow in the intestinal tract, thus producing more bacterial cells containing additional amounts of the enzyme Beta-galactosidase.

Gilland (1987) described, the desired effects of the lactobacilli in the host animals as follows :-

a) Inhibiting the growth of intestinal pathogens.

b) Providing key enzymes such as Beta-galactosidase for lactose hydrolysis or enzymes to act on other substances.

c) Assimilating cholesterol.

d) Increasing or improving growth and performance of livestock.

Johnes and Thomas (1987) recommended the criteria for an effective probiotic as follows :

1) It should be non pathogenic.

2) It should be Gram positive.

3) It should be acid resistant-acid producer.

4) It should be strain specific.

5) It should liberate anti E.coli factor.

6) It should be bile resistant.

7) It should be viable/stable.

Fuller (1989) suggested that fermented milk contained bacterial metabolites which inhibits cholesterol synthesis in the body. However some lactobacilli have a direct effect on cholesterol level by assimilation and removal from the growth medium.

Gandhi and Nagarathnam (1990) stated that the spores of *Lactobacillus sporogenes*, are nature's own most powerful microencapsulation system (i.e. Calcium dipicoline, acid-peptidoglycan complex) endowed to resist heat, desication, gastric acidity, bile acids of duodenum and posses anti-coliform and anti-salmonella activity. Thus they posses all characteristics of an ideal probiotic for ruminant use. These probiotics while regulating or enhancing the microbial environment allow the establishment of healthy rumen-microflora, and reduced digestive upsets, improve feed utilisation and increases animal performance.

YEAST

Yeast culture is a dry product composed of yeast and the media on which it is grown and dried in such a manner as to preserve the fermenting capacity of yeast.

Rose (1987) stated that, structurally, yeast has a thick polysaccharide containing wall which encloses a protoplasm that closely resembles the animal and plant cell. Chemically, yeast is made up of about 40% protein, 15% nucleic acid (DNA and RNA), 25% polysaccharide and 15% fat or lipid, the remainder is accounted for water soluble compounds such as nucleotides, aminoacids, vitamins and minerals.

Newman and Dawson (1987) reported that feeding of live yeast culture, raised the rumen pH from 6.36 to 6.55.

Wu (1987) suggested that the activity of the yeast culture may be associated with the following :

1) The yeast posseses a natural attractive flavour which can improve the palatability of the food.
2) Yeast contains B-complex vitamin and unknown growth factors both of which may be essential for the nutrition of specific gastrointestinal micro-organisms and for host animal metabolism.
3) Yeast assimilates many proteins and secretes many essential amino acids.

4) Yeast provides minerals which are available in chelated form and are liberated after autolysis of yeast cells. Such minerals are more readily absorbed by the animal.
5) Yeast secretes digestive enzymes including protease, lipase, proteinase, and invertase.
6) Yeast also produces ergosterol, sterols, lipids, glycolipids, some polypeptides.
7) During fermentation, yeast produces acetate which is the precursor of fat synthesis.

Thomas (1987) found that the inclusion of Yea-Sacc (*Saccharomyces cerevisiae*) in the diet of lactating cows can lead to better and efficient utilisation of feed nutrients for production of milk.

Dawson and Newman (1987) and Harrison *et.al.* (1987) found that, inclusion of yeast culture increases bacterial numbers, leading to increased microbial protein supply to the cow, which may account for positive effects on milk protein, when Yea-Sacc (*Saccharomyces cerevisiae*) is included in the ration.

Dawson (1987) found that, the inclusion of yeast culture produced 5 fold increase in the counts of anaerobic bacteria and doubled the number of cellulolytic bacteria.

Petersen *et al* (1987) found that, the live yeast cultures have the ability to chelate minerals and render them more biologically available to animals. Since several minerals are integral parts of enzyme systems or are involved in nutrient metabolism in direct manner, increased mineral availability would increase feed utilisation and efficiency.

Dildey (1988) observed that, the inclusion of high strength, viable yeast cultures Yea-Sacc (*Saccharomyces cerevisiae*, Alltech Inc.) in lactating dairy cow diets can result in icreased milk yield upto 10% with similar results in milk proteins and butter fat yields. He further noted that live yeast culture will secrete enzymes and vitamins that will supplement those being synthesised by the animal and will improve overall animal digestive performance.

2.5.2 *RUMENOTORIC.*

Fraser *et.al.*(1986) defined rumenotorics as the agents and mixtures that promote forestomach function (fermentation and motility). Generally such mixtures consist of bitters (eg. nux vomica, ginger, capsicum, gentian) to stimulate salivation and perhaps ruminal contractions, alkalinizing compounds (eg. magnesium oxide and hydroxide, magnesium trisilicate, calcium and ammonium carbonate, sodium bicarbonate) to elevate a low ruminal fluid pH, Sodium phosphate to act as cathartic; glucogenic subtracts (eg. sodium or calcium propionate, glycerol, propylene glycol,

dextrose), minerals as co-factors for microbial enzyme function (eg. cobalt, copper, manganese, zinc, iron, calcium) and salts as buffers to maintain osmolarity (eg. phosphates).

2.5.3 *HERBAL PREPARATION.*

It is a combination of a selected herbs and salts which act as rumenotoric, and help in growth and multiplication of ruminal protozoa and bacteria.

Chopra (1958) reported that, *Picrorhiza kurrooa* is a bitter tonic almost as efficacious as gentian. It acts as antiperiodic and cholagogue. *Zingiber officinale* is a carminative and an aromatic stimulant. It is frequently given with purgatives to reduce griping. *Andrographis paniculata* also acts as a bitter. *Trachyspermum ammi* is a powerful antiseptic and aromatic carminative, tonic and antispasmodic.

Jain (1968) reported that, leaves and fruits of *Piper nigrum* have an antibacterial activity.

Material And Methods

MATERIAL AND METHODS.

The present study was undertaken at Unit No. 22, Aarey Milk Colony, Goregaon, belonging to Konkan Krishi Vidyapeeth and at various commercial buffalo farms in and around Bombay (Malad, Chembur, Thane, Mulund, Digha etc.). The study included the observations on 32 milch buffaloes of murrah breed and of different age (Appendix I). Of these, 24 animals suffering from anorexia were arbitrarily divided into three groups (A, B and C) of eight animals each. The remaining eight animals were apparentely healthy and served as control group (D).

Group A : were subjected to treatment with a probiotic (Bioboost).

Group B : were subjected to treatment with a rumenotoric (Floratone).

Group C : were subjected to a treatment with a herbal preparation (Rumbion).

(Plate 3.1)

Group D : control.

The study was conducted during the period from August to December 1990.

PLATE : 3.1 : MEDICINES USED IN THE TREATMENT OF ANOREXIA.

a) BIOBOOST BOLUS.
b) FLORATONE BOLUS.
c) RUMBION BOLUS.

3.1 SELECTION AND OUTLINE OF CASE STUDY.

Animals suffering from anorexia were selected for the present study. Secondary anorexia cases were differentiated by various methods of physical examination (Rosenberg, 1979), blood examination (Coles, 1980 and Benjamin, 1985) and urine examination for pathological constituents (Benjamin, 1985).

3.2 DIAGNOSTIC PROCEDURE

A) *History*

For uniformity in collecting the data a questionaire was prepared (Appendix II) and individual case history was obtained in detail from the owner. These were systematically recorded in a format designed for this purpose.

B) *Rumen Fluid.*

1) *Collection.*

Collection of rumen fluid was made with the help of 'Rumen Fluid Extraction Pump ' (Ravel Scientific Instruments, Madras). Each animal was restrained properly. The tube was lubricated with liquid paraffin and the animals head was held high enough to facilitate the intubation and the tongue was pulled out to one side. Some resistance was felt as it reached the cardia and it was passed further until a firm resistance was felt indicating that it had reached the

ventral sac of the rumen. About 150-200 ml of rumen fluid was collected by suction. Then the tube was gently withdrawn (Plate 3.2).

2) ***Rumen Fluid Evaluation***

a) Colour, odour and consistency of the rumen fluid was observed as per the method of Rosenberg (1979).

b) Hydrogen ion concentration (pH) was measured immediately after collection of rumen fluid, with a pH meter (pocket type) [Tekno Corporation, Hyderabad].

c) Sedimentation Activity Test (SAT) was done as per Nichols and Penn (1958).

d) Methylene Blue Reduction Time (MBRT) was noted as described by Dirksen (1983).

e) Protozoal Motility

A drop of freshly collected ruminal fluid was taken on a slide topped with a cover slip, protozoal motility was observed under low power and recorded as +1, +2, +3 or +4.

f) Protozoal density was observed under low power field (LPF) and graded as follows :

++++	Vigorous	
+++	Abundant	: More than 30 protozoa/LPF
++	Moderate	: 10-30 protozoa/LPF
+	Few	: 1-10 protozoa/LPF
-	None	: --

PLATE : 3.2 : COLLECTION OF RUMEN FLUID FROM A BUFFALO.

g) Proportion of dead to live protozoa :

0.2% trypan blue (Lobo-Chemie Industrial Co. Bombay-5) solution was used for this purpose, with slight modification of the trypan blue exclusion technique as described by Jagadish, 1977.

Procedure : 0.1 ml of Strained Rumen Liquor (SRL) was diluted with 0.9 ml of 0.2% Trypan blue solution. After mixing, one drop was taken on a glass slide and a cover slip was placed on it. It was then observed under low power objective. The live protozoa do not take up the stain whereas the dead cells are stained blue. The live and dead protozoa were counted in a minimum of 15 fields (LPF) were counted and the proportion of dead to live protozoa was then calculated.

h) Iodophilic activity of protozoa : A drop of SRL was taken on a slide and one drop of Lugol's iodine (Impero Diagnostic Reagents, Bombay) was placed over it and covered with a coverglass and examined under low power objective. Iodophilic activity of protozoa can be recognised by black colouration of starch contents in the protozoa. Iodophilic activity was graded as 0, +1, +2. +3, +4 depending on the quantity of the starch content.

i) Total protozoal count : was done by the modified method of Sankaranarayan and Nambiar.(1972)

j) Total bacterial count was done as per the method described by Gall *et al* (1949).

3.3 HAEMATOLOGICAL STUDIES :

Five ml of blood collected from each animal in a vial containing 0.5 ml of Heller's and Paul's double oxalate solution. Haemogram was carried out as per Benjamin, 1985 and Jain, 1986.

For serum biochemical studies, ten ml of blood was collected in a test tube, allowed to clot and serum was seperated. The sera samples were stored at -20^{o}C until used (Benjamin, 1985).

For blood glucose, five ml of blood was collected in a test tube containing 40 mg of Fluoride-oxalate solution (Benjamin, 1985). Blood glucose was estimated as per orthotoludine method of Dubowski (1962) by using glucose kit.

Serum glutamic oxaloacetic-transaminase (SGOT)/Aspartate aminotransferase was determined by using commercially available diagnostic kit. This test was performed as outlined by Reitman and Frankel (1957).

Serum cholesterol was determined by using commercially available diagnostic kit. This test was performed as outlined by Wooton (1964).

3.4 URINALYSIS

Urine samples were collected and subjected to pH, Sulkowitch and Rothera's test (Benjamin, 1985).

3.5 TREATMENT

Table No. 3.1.

Details of treatment.

Group	No. of animals	Drug used	Dose	No. of Treatments
A	8	Probiotic	2 boli BID	3 - 5
B	8	Rumenotoric	4 boli BID	3 - 5
C	8	Herbal Preparation	2 boli BID	3 - 5
D	8	--	--	--

3.5.1 *PROBIOTIC (Bioboost bolus)*

Each bolus of 6.1570 gms provides -

Live Yeast Culture	3 gm
Amino Acids	2 mgm
Live *Lactobacillus Sporogenes* Culture	20 million CFU
Liver extract	5 mg
Excipient	Q.S.

3.5.2 *RUMENOTORIC (Floratone bolus)*

Each bolus of 3.6886 gms provides

Methionine	40.00 mg
Cobalt Sulphate	0.88 mg
Copper Sulphate	0.44 mg
Sodium Phosphate (Dibasic Dihydrate)	100.00 mg
Sodium Bicarbonate	660.00 mg
Magnesium Trisilicate	1000.00 mg
Gentian Powder	220.00 mg
Ginger Powder	44.00 mg
Vitamin B_1	145.00 mg
Nicotinamide	165.00 mg
Dried Yeast	700.00 mg
Dextrose	500.00 mg

3.5.3 *HERBAL PREPARATION*

Each bolus of 5.00 gms

Rumbion contains herbal ingredients like *Picrorhiza kurrooa, Zingiber officinale, Andrographis paniculata, Trachyspermum ammi, Piper nigrum.*

3.6. STATISTICAL ANALYSIS.

The statistical analysis of the data was carried out as per Snedecor and Cochran (1967). The difference between the groups for rumen fluid parameters, haematalogical and biochemical parameters were studied by analysis of variance for one way classification. Effect of treatment within the group was studied by paired " t " test.

0 bservations

OBSERVATIONS

This study included the observations on 32 milch buffaloes (Murrah) between 7 to 10 years of age. Of these 24 were suffering from anorexia (20 cases of primary anorexia and 4 from secondary anorexia) and were arbitrarily divided into three groups as A, B and C consisting of 8 animals in each group. The remaining 8 were selected from apparently healthy animals and served as controls (group D).

4.1 DIAGNOSIS

4.1.1 CLINICAL EXAMINATION :-

History :- The results of clinical examination and observations on rumen fluid are presented in table 4.1. Temperature, respiration rate and pulse rate were found to be within normal range except one case of group A (secondary anorexia) which showed increased temperature (105^{o}F), respiration rate (26/min) and pulse rate (78/min). Ruminal motility was decreased in all the animals suffering from anorexia and was ranging between 1 per 2.5 to 3 minute whereas it was 1 to 3 per minute in the control group of animals.

4.1.2 RUMEN FLUID :-

1) Collection of rumen fluid :-

Rumen fluid sample from each animal was collected with the help of "Rumen Fluid Extraction Pump". This method was found most satisfactory and easy when the animal was restrained properly. About 150 to 200 ml of rumen fluid sample was collected from each animal (anorectic and healthy)

2) Observations on rumen fluid :-

a) Colour : Rumen fluid was found to be brownish green, brown , dark brown, light, brown or greenish brown in colour in all the animals including controls.

b) Odour : Slight acid odour was noted in animals with acid indigestion, ammoniacal to putrid in alkaline indigestion and aromatic to ammoniacal animals with non-specific anorexia. Slight acid to aromatic odour was smelt in animals of control group.

c) Consistency : Watery to slight viscous consistency of rumen fluid was observed in all the animals of groups A, B, C and D.

d) pH : Out of the 24 animals of group A, B and C suffering from anorexia, the pH range of 6.0 to 7.0 and 7.1 to 8.5 was observed in 3 and 21 cases respectively. A pH range of 6.0 to 7.0 was observed in animals of group D. (Plate 4.1).

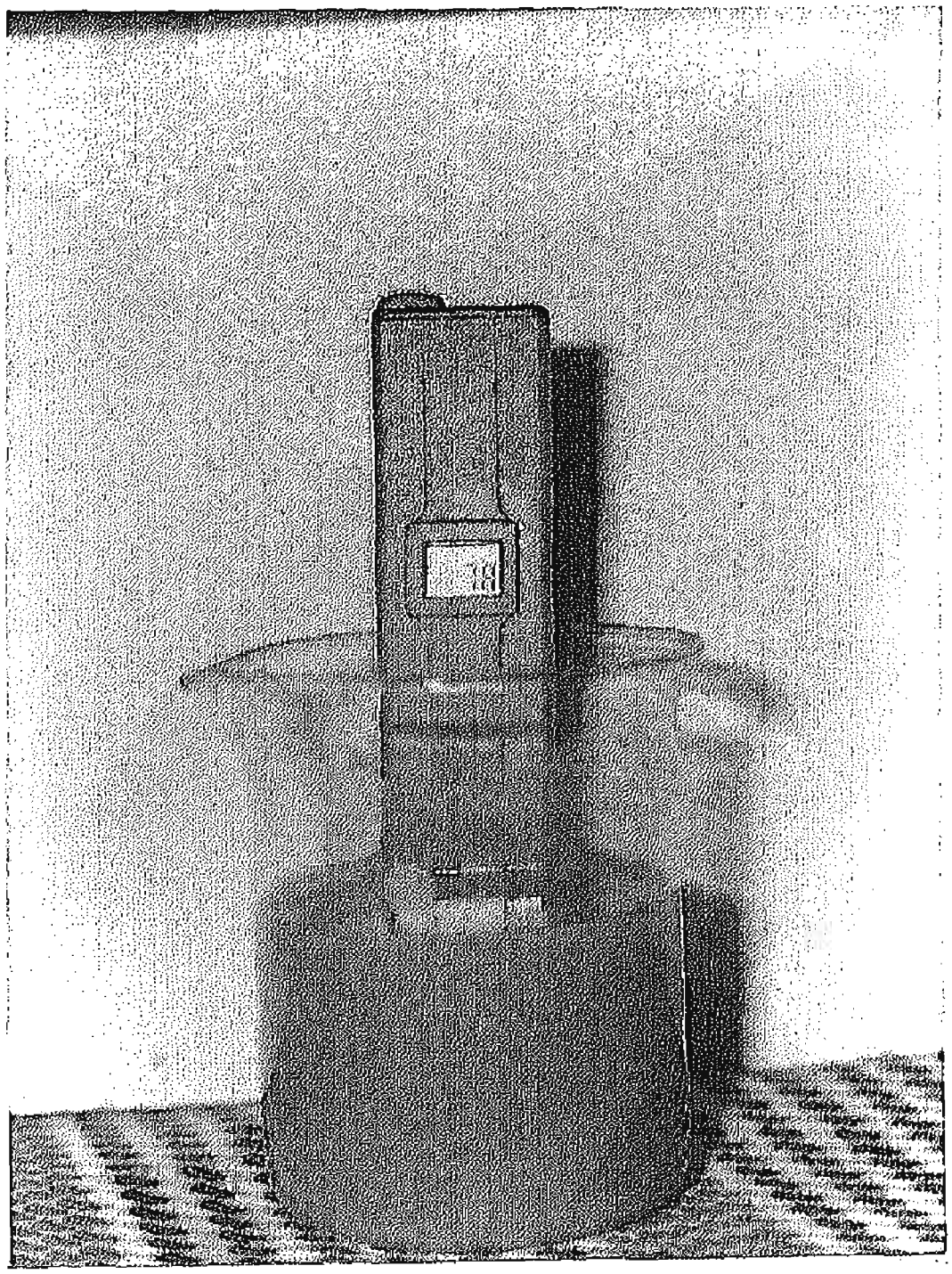


PLATE : 4.1 : RECORDING OF RUMEN FLUID pH WITH THE HELP OF pH METER (Pocket type).

e) Sedimentation activity test (SAT) : An increase in SAT was observed in animals of groups A, B and C in all anorectic animals. It was ranging between 4 to 19, 8 to 11 and 4 to 9 minutes respectively with means of 11.75 $\pm$ 1.60, 9.38 $\pm$ 0.42 and 7.50 $\pm$ 0.63 minute respectively. Two cases of group A recorded more time to sediment (15 and 19 minutes) while range of 5 to 6 minutes with a mean of 5.50 $\pm$ 0.19 minute was observed in group D. (Plate 4.2).

f) Methylene blue reduction time (MBRT) : MBRT was found to be increased (3 to 20 minutes) in all animals suffering from anorexia of groups A, B and C whereas in group D a range of 3 to 5 minute was observed. The mean values of MBRT were 11.38 $\pm$ 1.71, 6.75 $\pm$ 1.15 and 6.75 $\pm$ 0.82 minutes in group A, B and C respectively. (Plate 4.3).

g) Protozoal motility : Motility of the protozoa was found to be decreased (+1 to +2) in all anorectic animals. Protozoal motility (+2 to +3) was observed in healthy animals.

h) Protozoal density : Density of the protozoa was found to be decreased in most of the cases (+1 to +2). Protozoal density (+2 to +4) was observed in healthy animals.

i) Proportion of dead to live protozoa : Proportion of dead protozoa was increased in all the anorectic animals with reduced live protozoal number. The mean live protozoal

PLATE : 4.2 : SEDIMENTATION ACTIVITY TEST/FLOATATION TIME. (SAT).

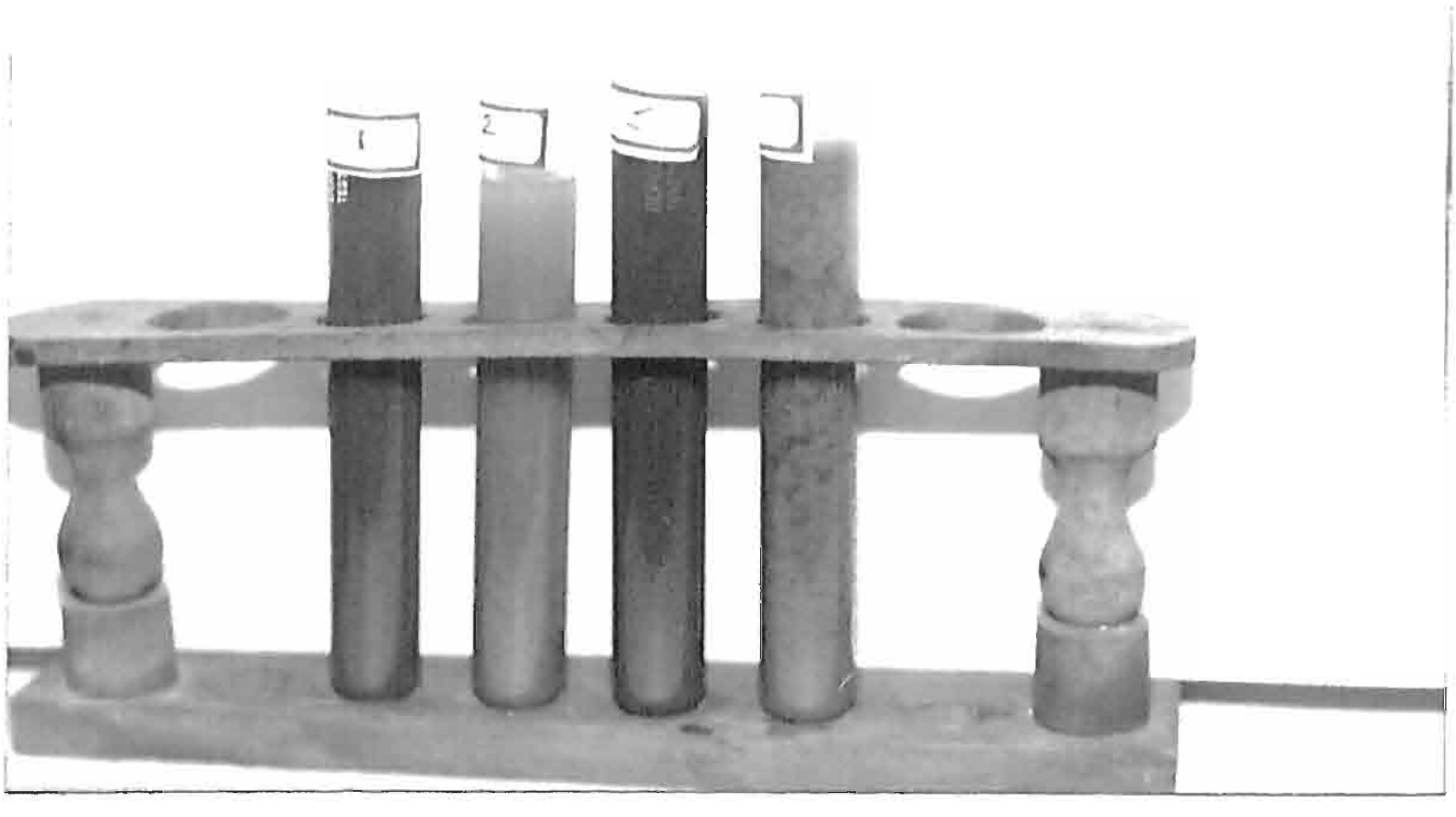


PLATE : 4.3 : METHYLENE BLUE REDUCTION TIME (MBRT) (BEFORE REDUCTION).

proportion per unit of dead protozoa was decreased in groups A, B and C (1.82 ± 0.63, 1.53 ± 0.76 and 1.85 ± 0.26 respectively), while it was 6.19 ± 1.09 in group D. (Plate 4.4)

j) Iodophilic activity of protozoa : Iodophilic activity of protozoa was decreased in all anorectic animals (+1 to +2) with relative increase in non-iodophilic protozoal proportion per unit of iodophilic protozoa in groups A, B and C (3.22 ± 0.92, 3.03 ± 0.68, 1.81 ± 0.46 respectively). Whereas in group D, it was 0.78 ± 0.15.

k) Total protozoal count (TPC) : There was decrease in total protozoal count in all anorectic animals. In groups A, B and C, the average count was $2.04 \pm 0.41 \times 10^5$ /ml, $1.91 \pm 0.33 \times 10^5$ /ml, $1.84 \pm 0.46 \times 10^5$ /ml of rumen liqour respectively. Whereas in group D the count was $3.82 \pm 0.46 \times 10^5$ /ml of rumen liqour.

l) Total bacterial count : There was decrease in total bacterial count in all anorectic animals. In group A, B and C the average count was $6.93 \pm 0.27 \times 10^8$ /ml, $6.56 \pm 0.11 \times 10^8$ /ml and $6.69 \pm 0.33 \times 10^8$ /ml of rumen liqour respectively). Whereas in group D, the count was $9.91 \pm 0.50 \times 10^8$ /ml of rumen liqour.

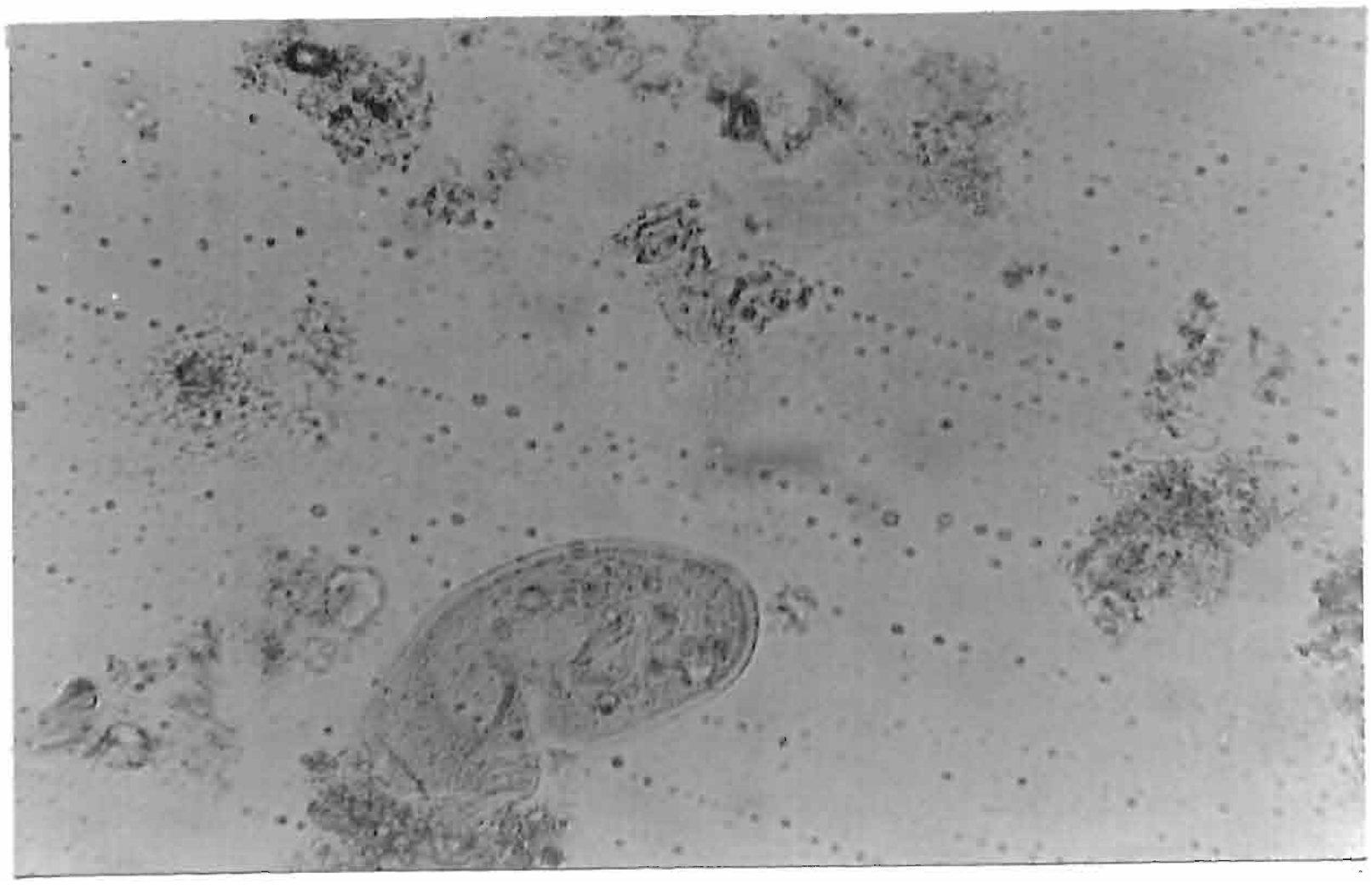


PLATE : 4.4 : PROTOZOA OF THE RUMEN FLUID.

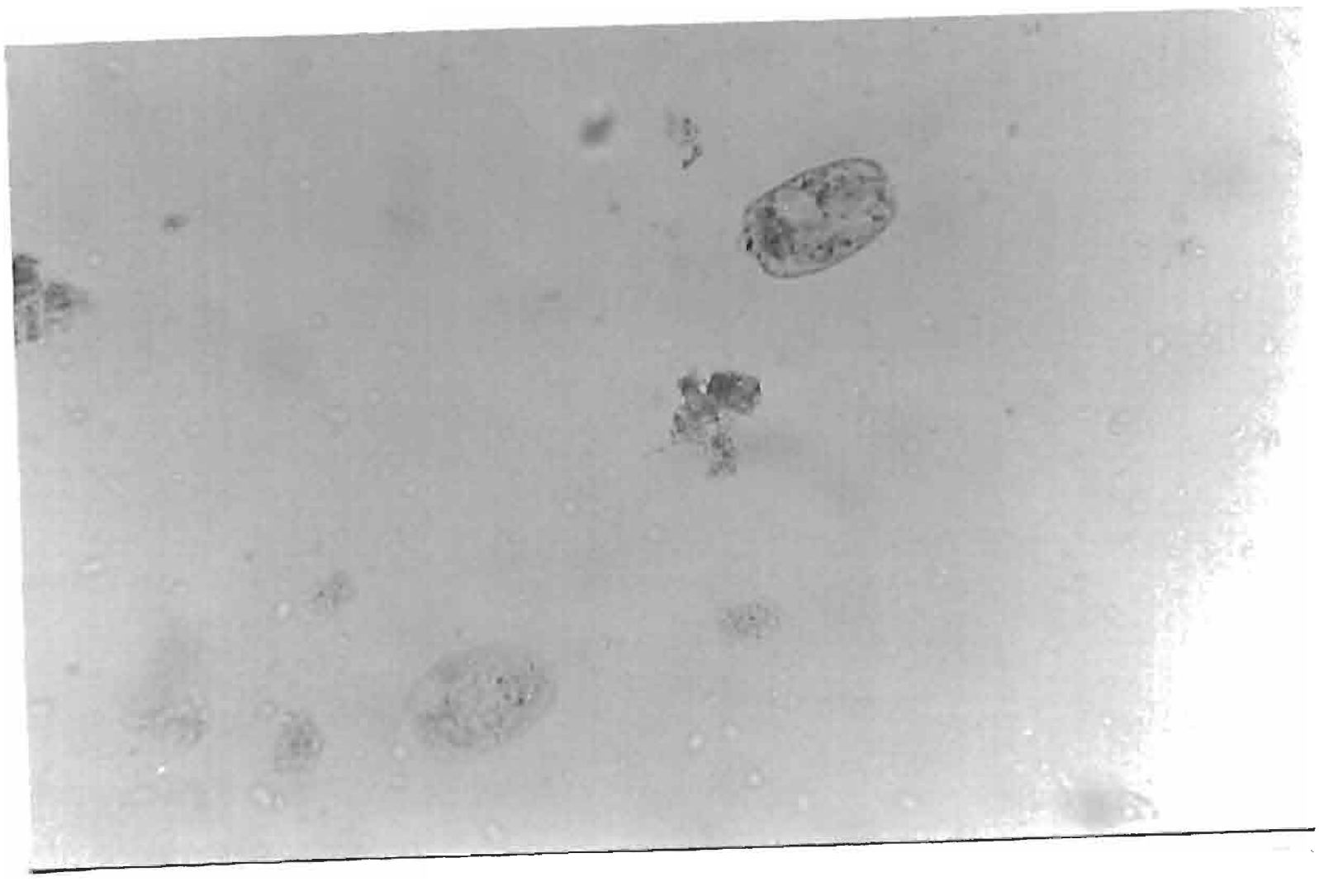


PLATE : 4.4 : DEAD (BLUE) AND LIVE (UNSTAINED) PROTOZOA OF RUMEN FLUID

4.2. HAEMATOLOGICAL AND BIOCHEMICAL STUDIES:-

4.2.1. HAEMATOLOGICAL STUDIES :

The results of haemogram studies and biochemical changes before and after treatment are presented in table 4.2. Hb and PCV values were found to be within normal range in all the groups.

Differential luekocyte count revealed neutrophilia, lymphocytopenia in all the anorectic animals compared with control animals. Eosinophil, monocyte and basophil percents in affected and control animals were within the normal ranges.

4.2.2. BIOCHEMICAL CHANGES :-

Mean blood glucose levels were considerably higher (70.48 $\pm$ 0.77, 72.53 $\pm$ 1.11 and 75.77 $\pm$ 1.92 mg/dL) in groups A, B and C than group D (61.93 $\pm$ 0.25 mg/dL).

Mean cholesterol levels were considerably higher (228.57 $\pm$ 5.50, 203.62 $\pm$ 7.55 and 203.30 $\pm$ 6.97 mg/dL) in groups A, B and C than group D (96.59 $\pm$ 4.19 mg/dL).

Serum glutamic oxaloacetic transaminase (SGOT) activity was increased in all anorectic buffaloes. The average levels of 60.04 $\pm$ 1.36, 58.33 $\pm$ 3.24 and 55.12 $\pm$ 2.80 I.U. /L in groups A, B and C respectively and 30.05 $\pm$ 0.69 I.U. /L in healthy animals of group D were recorded.

Table 4.1

Clinical examination and observation on rumen fluid parameters of buffaloes suffering from anorexia (Groups A, B and C.) before treatment and apparently healthy animals (Group D).

Group A.

Case No.	History	Clinical signs	Temp. °F	Resp. /min	Pulse /min	Rumen mot./ min	Colour	Odour	Consis tency	pH	SAT (min)	MBRT (min)	PROTOZOA Mot.	PROTOZOA Den.	PROTOZOA TPC $x10^5$ /ml	PROTOZOA NIP. /UIP	PROTOZOA LP. /UDP	Total BC 10^8 /ml
1.	Anorexia since two days	Dull, rough hair coat	102.8	22	78	3.0	Dark brownish black.	Putrid	Slight viscous	8.0	13	14	+1	+1	4.22	0.86	0.52	6.53
2.	Anorexia since one day	Dull, emaciated	101.2	17	66	2.5	-do-	-do-	-do-	8.5	15	12	+1	+1	0.77	6.00	1.25	6.23
3.	Anorexia, refusal of conc.	Reduced rumination, dull	101.0	16	62	2.5	Brown	Slight putrid	-do-	8.0	19	20	+1	+1	1.66	2.66	0.69	6.83
4.	Anorexia since three days, aborted, mastitis	Dull, congested mucous membrane, rapid respiration, rough haircoat	105.0	26	78	3.0	Dark Brown	-do-	-do-	8.0	10	12	+1	+1	2.88	1.57	0.52	6.07
5.	Anorexia since two days, refusal of conc.	Dull, rough hair coat	101.0	16	66	3.0	Brown	-do-	Watery	8.5	4	5	+2	+2	1.78	8.2	4.00	7.5
6.	Anorexia since two days	Dull, rough hair coat	100.0	18	62	3.0	Brown	Ammoniacal	-do-	8.0	14	12	+1	+1	2.67	2.66	0.67	6.4
7.	Inappetence since two days	Reduced rumination, dull, rough haircoat	102.6	20	63	3.0	Brown	-do-	-do-	8.0	9	11	+1	+1	1.11	2.94	1.77	7.84
8.	Anorexia since two days	Reduced rumination, dull	100.0	16	65	2.5	Light brown	Slight putrid	-do-	7.5	10	5	+2	+2	1.22	0.86	5.13	8.0

Table 4.1 Contd.
Group B.

Case No.	History	Clinical signs	Temp. °F	Resp. /min	Pulse /min	Rumen mot./ -min	Colour	Odour	Consis tency	pH	SAT (min)	MBRT (min)	PROTOZOA Mot.	Den.	TPC $x10^5$ /ml	NIP. /UIP	LP. /UDP	Total BC 10^8 x /ml
1.	Anorexia since two days	Dull, rough hair coat, reduced rumination	101.0	18	64	3.0	Dark brown	Slight acidic	Watery	6.5	11	5	+1	+1	0.88	5.42	1.33	6.40
2.	Inappetence since 4 days	Dull, rough hair coat	101.0	20	63	3.0	Brownish green	-do-	-do-	6.5	11	5	+2	+2	1.88	1.4	6.69	6.47
3.	Anorexia since 2 days	--do--	101.2	17	65	3.0	Brown	Ammon-iacal	-do-	7.8	8	3	+1	+1	1.0	2.94	0.4	6.83
4.	Inappetence, refusal of conc.	Dull	101.8	20	62	3.0	-do-	-do-	Slight viscous	8.0	9	7	+1	+1	3.33	6.43	1.77	6.67
5.	Inappetence, refusal of conc.	Sweet odour of breath, suspected for ketosis	102.6	21	63	3.0	Light brown	-do-	-do-	8.0	10	14	+1	+1	1.11	1.5	0.4	6.3
6.	Anorexia since 3 days	Dull, reduced rumination	101.0	19	62	2.0	Dark brown	-do-	-do-	7.9	9	7	+1	+1	2.89	1.63	0.52	6.43
7.	Anorexia since 2 days, refusal of conc.	Dull, rough hair coat	101.4	17	64	2.0	Brown	Aroma-tic	Watery	7.6	8	6	+1	+1	1.67	1.89	0.64	7.12
8.	Anorexia since 2 days refusal	Dull, rough	102.4	23	66	2.5	Dark	-do-										

Table 4.1 Contd.
Group C.

Case No.	History	Clinical signs	Temp. °F	Resp. /min	Pulse /min	Rumen mot./ -min	Colour	Odour	Consis tency	pH	SAT (min)	MBRT (min)	PROTOZOA Mot.	PROTOZOA Den.	PROTOZOA TPC $x10^5$ /ml	PROTOZOA MIP. /VIP	PROTOZOA LP. /UDP	Total BC 10^8 /ml
1.	Anorexia since 2 days	Dull	101.2	16	66	3.0	brown	Slight putrid	Watery	7.8	9	6	+1	+1	1.66	2.66	0.52	5.87
2.	Anorexia since 2 days	Dull, rough hair coat	101.6	18	64	3.0	-do-	Putrid	Slight viscous	8.0	4	4	+1	+1	0.88	0.58	1.87	5.3
3.	Inappetence refusal of conc. since 1 month.	Dull, emaciated	102.0	16	60	3.0	Dark brown	Slight putrid	watery	7.4	9	9	+1	+1	1.33	0.92	2.89	6.51
4.	Anorexia since 4 days	Dull, rough hair coat	101.0	18	65	2.0	-do-	-do-	-do-	8.0	6	5	+2	+2	4.88	0.43	2.52	6.07
5.	Anorexia since 1 day	Dull, rough hair coat	101.0	17	64	3.0	Light brown	-do-	-do-	7.0	8	5	+1	+1	1.7	1.38	2.05	8.23
6.	Anorexia since 1 day	Dull, rough hair coat	101.0	16	62	3.0	Brown	Putrid	Slimy	8.5	9	11	+1	+1	2.22	4.19	1.85	7.0
7.	Anorexia since 2 days	Dull	101.2	17	60	3.0	-do-	Slight putrid	Watery	8.0	7	7	+1	+1	0.89	2.79	1.25	7.33
8.	Anorexia since 1 day	Dull, rough hair coat	101.6	16	62	3.0	-do-	-do-	-do-	7.6	8	7	+1	+1	1.11	1.5	1.87	7.20

Table 4.1 Contd.
Group D.

Case No.	History	Clinical signs	Temp. °F	Resp. /min	Pulse /min	Rumen mot./-min	Colour	Odour	Consis-tency	pH	SAT (min)	MBRT (min)	PROTOZOA Mot.	Den.	TPC. $x10^5$ /ml	NIP. /UIP	LP. /UDP	Total BC. 10^6 x /ml
1.	--	--	101.4	17	45	3	Brownish green	Slight acid	Slight viscous	6.8	6	4	+3	+4	5.44	0.37	11.5	9.92
2.	--	--	101.4	17	47	3	Green	Aroma-tic	-do-	7.0	6	3	+3	+3	5.55	0.18	9.67	7.33
3.	--	--	102.0	20	50	2	Brown	Slight acid	-do-	6.7	6	5	+3	+2	2.33	1.11	4.8	8.43
4.	--	--	101.2	16	45	2	-do-	Aroma-tic	Watery	6.9	5	5	+3	+2	2.67	1.26	3.06	9.49
5.	--	--	101.4	22	52	3	Greenish brown	-do-	Slight viscous	7.0	5	4	+2	+3	4.88	1.12	7.57	10.83
6.	--	--	101.2	17	52	2	-do-	-do-	-do-	7.1	6	3	+2	+3	2.67	0.67	5.0	11.25
7.	--	--	100.8	16	54	3	Brown	-do-	Watery	7.0	5	4	+3	+4	3.44	0.38	3.44	11.0
8.	--	--	102.0	16	55	3	-do-	Ammon-iacal	-do-	7.0	5	5	+3	+4	3.56	1.11	4.44	9.25

Temp. - Temperature.
Resp. - Respirations.
Mot. - Motility.
SAT. - Sedimentation activity test.
MBRT. - Methylene blue reduction time.
Den. - Density.

TPC. - Total protozoal count.
NIP. - Non iodophilic protozoa.
UIP. - Unit of iodophilic protozoa.
LP. - Live protozoa.
UDP. - Unit of dead protozoa

TABLE No. : 4.2

HAEMATOLOGICAL AND BIOCHEMICAL VALUES OF BUFFALOES SUFFERING FROM ANOREXIA (GROUPS A, B, AND C) BEFORE AND AFTER TREATMENT AND APPARENTLY HEALTHY ANIMALS (GROUP D).

GROUP A.

Case No.	BEFORE TREATMENT												AFTER TREATMENT											
	Hb gm %	PCV %	TRBCs x 10^6	TWBCs x 10^3	DLC(%) N	L	E	M	B	Biochemistry Bg. mg/dL	Chol. mg/dL	SGOT I.U./L	Hb gm %	PCV %	TRBCs x 10^6	TWBCs x 10^3	DLC(%) N	L	E	M	B	Biochemistry Bg. mg/dL	Chol. mg/dL	SGOT I.U./L
1.	17.4	42	6.50	9.35	47	49	2	2	0	68.46	255.38	62.2	17.2	41	6.23	8.40	42	51	4	2	1	63.85	200.00	53.20
2.	13.4	38	5.23	5.90	50	42	6	1	1	72.31	218.46	60.0	13.6	38	5.50	6.20	43	49	4	3	1	62.31	154.55	43.44
3.	10.8	44	3.92	10.00	72	26	2	0	0	69.23	236.92	59.0	11.0	43	4.00	8.85	43	41	8	8	0	64.62	200.00	48.72
4.	13.0	29	3.50	8.00	50	42	6	2	0	74.62	236.92	63.2	13.2	30	3.72	7.80	45	46	4	4	1	63.08	170.00	53.52
5.	13.0	48	4.86	8.90	48	43	5	4	0	70.00	221.53	52.32	13.2	48	4.80	8.50	46	49	3	2	0	62.31	168.18	41.52
6.	13.4	48	9.27	5.10	48	44	5	2	1	68.46	213.16	57.36	13.4	47	7.70	5.20	44	49	4	3	0	61.54	181.81	51.60
7.	14.4	38	3.20	7.50	50	45	4	0	1	69.23	209.23	64.1	14.4	39	4.00	6.55	44	49	4	3	0	63.85	159.09	58.32
8.	13.0	40	3.80	6.20	48	45	4	3	0	71.54	236.92	62.15	13.4	40	4.00	6.20	46	48	3	2	1	60.77	204.55	40.32
	GROUP B																							
1.	13.4	38	4.21	6.00	57	40	3	1	0	74.62	221.16	53.2	13.2	39	4.21	6.00	50	44	4	1	1	64.62	181.81	46.96
2.	13.2	46	6.80	6.40	52	46	2	0	0	73.08	181.81	53.52	13.4	45	6.20	6.40	49	45	5	1	0	66.15	140.91	49.77
3.	14.2	46	7.10	7.00	49	46	4	1	0	68.46	195.45	58.52	14.0	45	6.80	6.90	44	50	4	1	1	60.00	150.00	49.96
4.	13.4	40	9.27	5.90	48	50	1	0	1	76.92	236.92	53.20	13.6	40	8.00	5.80	45	50	3	2	0	62.31	190.91	47.90
5.	14.0	48	4.86	8.75	52	38	8	2	0	62.20	190.90	44.31	13.4	48	4.60	9.00	56	32	6	6	0	75.00	136.36	31.76
6.	13.6	44	6.82	6.00	51	44	3	1	1	71.54	209.23	68.60	13.6	43	6.72	6.00	46	49	3	2	0	60.00	163.64	62.16
7.	13.2	46	6.22	5.80	56	44	4	2	0	70.77	175.00	63.20	13.4	45	6.22	5.90	45	50	4	1	0	63.85	145.91	51.65
8.	13.2	46	6.40	7.10	48	50	2	0	0	72.31	218.46	72.00	13.4	45	6.30	7.00	46	51	2	2	1	62.31	195.45	57.26

TABLE No. : 4.2 (Contd)

GROUP C.

Case No.	BEFORE TREATMENT												AFTER TREATMENT											
	Hb gm %	PCV %	TRBCs x 10^6	TWBCs x 10^3	DLC(%) N	L	E	M	B	Biochemistry Bg. mg/dL	Chol. mg/dL	SGOT I.U./L	Hb gm %	PCV %	TRBCs x 10^6	TWBCs x 10^3	DLC(%) N	L	E	M	B	Biochemistry Bg. mg/dL	Chol. mg/dL	SGOT I.U./L
1.	13.4	41	5.27	5.90	40	50	4	6	0	48.50	218.46	54.46	13.2	40	5.00	5.80	44	46	4	6	0	73.00	159.09	33.40
2.	14.0	36	5.78	6.30	48	45	4	3	0	72.31	190.90	65.76	14.0	40	5.70	6.00	47	47	3	2	1	63.85	127.27	48.72
3.	12.4	42	5.24	8.40	57	38	2	3	0	48.00	180.00	38.26	12.0	42	5.11	8.20	58	37	3	2	0	62.40	154.55	31.41
4.	13.6	38	6.25	9.80	50	42	5	3	0	84.62	209.23	53.76	13.6	39	6.10	9.80	46	48	4	2	0	65.38	163.64	44.64
5.	14.2	42	7.20	10.60	51	46	3	0	0	75.38	195.45	60.00	14.2	42	6.80	10.10	43	51	3	2	0	62.31	136.36	46.32
6.	13.0	40	6.20	8.70	51	45	3	1	0	73.08	213.64	58.32	13.0	40	6.00	8.80	47	51	2	0	0	64.62	181.81	49.65
7.	12.8	41	5.60	7.55	49	46	4	1	0	72.31	236.92	57.00	13.0	40	5.60	7.45	44	49	3	3	1	66.15	177.27	44.40
8.	11.0	39	3.72	9.20	47	49	3	1	0	76.92	181.81	53.36	11.4	40	4.15	9.00	44	50	4	1	1	63.85	150.00	44.40
GROUP D																								
1.	12.0	34	6.00	6.20	46	49	3	2	0	61.54	86.36	30.72	-	-	-	-	-	-	-	-	-	-	-	-
2.	11.8	32	6.10	9.50	45	52	3	1	1	62.31	100.00	32.64	-	-	-	-	-	-	-	-	-	-	-	-
3.	10.6	39	5.85	11.00	46	48	4	2	0	63.08	90.91	30.05	-	-	-	-	-	-	-	-	-	-	-	-
4.	12.0	45	5.82	6.00	44	50	5	1	0	62.31	109.09	32.86	-	-	-	-	-	-	-	-	-	-	-	-
5.	10.8	32	5.22	6.00	45	48	4	2	1	61.54	61.82	28.56	-	-	-	-	-	-	-	-	-	-	-	-
6.	12.4	38	6.20	10.60	44	52	2	2	0	60.77	104.55	27.48	-	-	-	-	-	-	-	-	-	-	-	-
7.	11.0	38	5.95	9.60	46	47	4	2	1	61.54	113.64	29.52	-	-	-	-	-	-	-	-	-	-	-	-
8.	12.0	40	6.14	8.20	44	51	4	1	0	62.31	86.36	28.56	-	-	-	-	-	-	-	-	-	-	-	-

Hb. - Haemoglobin.
PCV. - Packed cell volume.
TRBCs. - Total red blood corpuscles.
TWBCs. - Total white blood corpuscles.
SGOT. - Serum glutamic oxalo transaminase.
Bg. - Blood glucose.
Chol. - Cholesterol.
DLC. - Differential leukocyte count.
N. - Neutrophil.
L. - Lymphocyte.
E. - Eosinophil.
M. - Monocyte.
B. - Basophil.

4.3. CLINICAL RESPONSE TO TREATMENT :-

The results of clinical response to treatment are given in table 4.3.

4.3.1. RUMEN MOTILITY :-

Rumen motility was observed to be improved in all groups (A, B and C) (2 to 3 per minute).

4.3.2. OBSERVATIONS ON RUMEN FLUID :-

a) Colour : Rumen fluid was found to be green, brownish green and brown in all recovered animals after treatment.

b) Odour : Slightly acidic, aromatic to ammoniacal odour was smelt in all recovered animals of group A, B and C.

c) Consistency : Rumen fluid was slightly viscous in all recovered animals however it was watery in one case of group A and 2 each in group B and C.

d) pH : A pH range of 6.7 to 7 was observed in all recovered animals except one case in group A and B which showed a ruminal pH of 7.6 and 7.1 respectively.

e) Sedimentation activity test (SAT) : SAT of 3 to 6 minutes was observed in all recovered animals except one case of group A in which it was 15 minutes. The average values of

SAT in groups A, B and C were 6.00 ± 1.34, 3.88 ± 0.35 and 4.63 ± 0.32 minutes respectively.

f) Methylene blue reduction time (MBRT) : MBRT was ranging from 3 to 14 minutes. Mean MBRT was found to be considerbaly reduced in groups A, B and C. The average values of MBRT were 6.38 ± 1.25, 3.75 ± 0.49 and 3.50 ± 0.27 respectively for groups A, B and C. (Plate 4.5).

g) Protozoal motility : Motility improved to +2 to +3 and +4 in 13 , 10 and 1 cases respectively in the treated animals.

h) Protozoal density : Protozoal density was increased in all the treated animals +2 to +3 except one case of group A, where it was +1.

i) Proportion of dead to live protozoa : The means of live protozoa per unit of dead protozoa were increased in groups A, B and C (3.89 ± 0.34, 3.72 ± 0.18 and 3.35 ± 0.20 respectively).

j) Iodophilic activity of protozoa : Iodophilic activity of protozoa increased with decrease in non-iodophilic protozoal proportion. Iodophilic activity was +2 to +3 in groups A, B and C. Mean proportion of non-iodophilic protozoal per unit of iodophilic were 1.28 ± 0.35, 1.35 ± 0.24 and 0.69 ± 0.16 in groups A, B and C respectively).

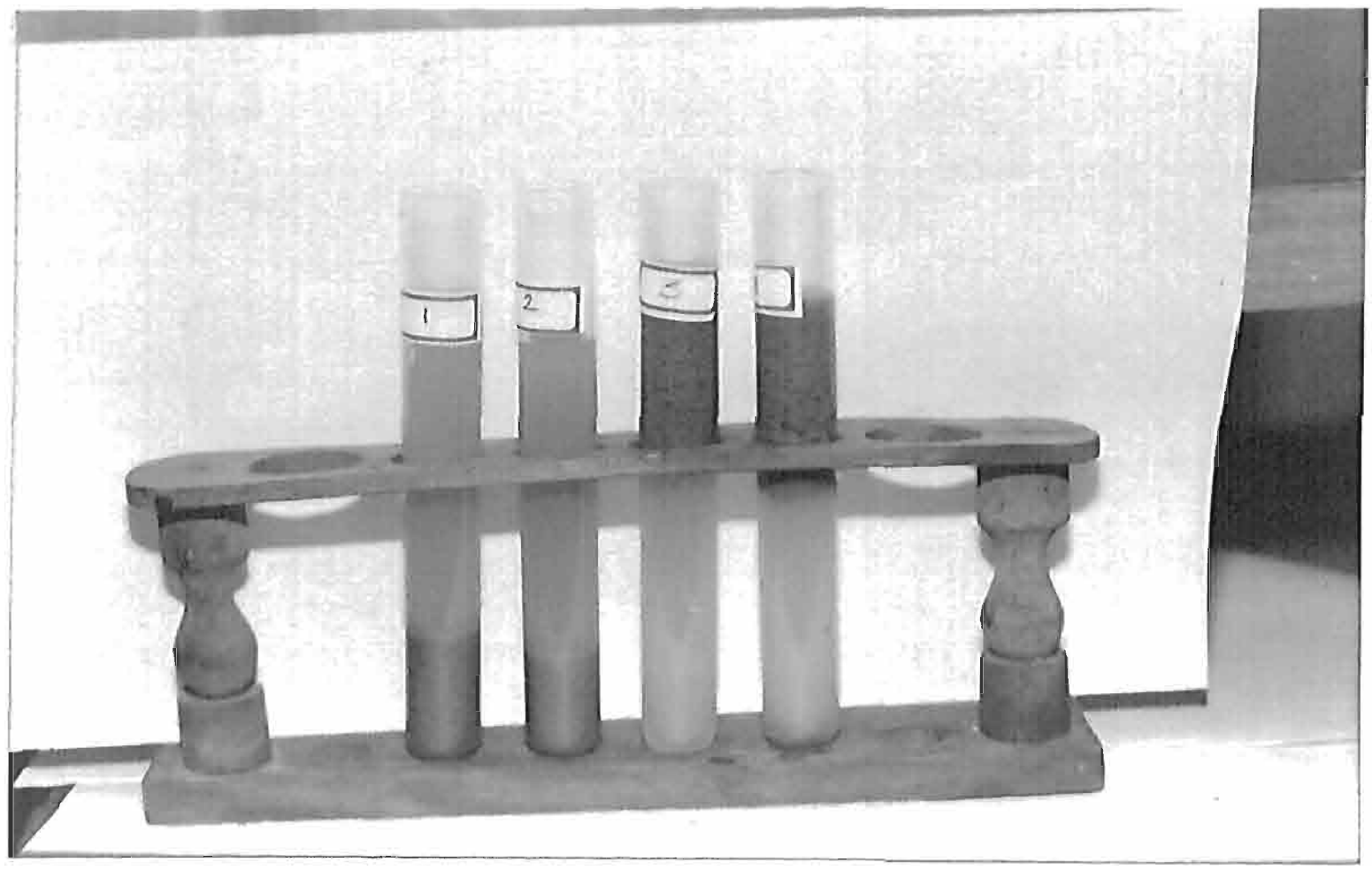


PLATE : 4.5 : METHYLENE BLUE REDUCTION TIME (MBRT)
(AFTER REDUCTION).

k) Total protozoal count (TPC) : TPC was increased in all the recovered animals of group A, B and C. The mean values of total protozoal counts were 3.89 $\pm$ 0.34 x 10^5/ml, 3.01 $\pm$ 0.28 x 10^5/ml and 2.88 $\pm$ 0.21 x 10^5/ml of rumen fluid in group A, B and C respectively.

l) Total bacterial count (TBC) : TBC was increased in all the recovered animals of group A, B and C. The mean values of bacterial counts were 8.18 $\pm$ 0.45 x 10^8/ml, 8.51 $\pm$ 0.38 x 10^8/ml and 8.31 $\pm$ 0.32 x 10^8/ml of rumen fluid in groups A, B and C respectively.

4.4. HAEMATOLOGICAL AND BIOCHEMICAL STUDIES :-

4.4.1. HAEMATOLOGICAL STUDIES :-

There was not much variation after treatment in Hb and PCV in all the groups. Neutrophil percentage was found to be decreased considerably (44.13 $\pm$ 0.52%, 46.43 $\pm$ 0.84% and 45.17 $\pm$ 0.70% as compared to pre-treatment values (51.63 $\pm$ 2.94%, 50.71 $\pm$ 1.19% and 49.50 $\pm$ 0.92 %) in groups A, B and C respectively. Lymphocyte percentage also returned to normal. No much variation was observed in eosinophil, monocyte and basophil percentages.

4.4.2. BIOCHEMICAL CHANGES :-

Considerable decrease in blood glucose (primary anorexia cases), cholesterol and SGOT levels were observed in treated animals.

The average biochemical values after treatment in groups A, B and C were (blood glucose 62.79 ± 0.46, 62.75 ± 0.94 and 64.36 ± 0.55 mg/dL, cholesterol 180.80 ± 6.88, 162.50 ± 8.49 and 156.25 ± 6.59 mg/dL and SGOT 46.52 ± 4.03, 49.22 ± 3.15 and 42.87 ± 2.39 I. U./L respectively) decreased and showing tendency to return to normal values. The treated animals showed improvement in appetite by 3rd to 5th day from the day of commencement of therapy.

Table 4.3

Clinical examination and observation on rumen fluid parameters of buffaloes suffering from anorexia (Groups A, B and C.) after treatment

Group A

Case No.	Temp. °F	Resp. /min	Pulse /min	Rumen cot./ min	Colour	Odour	Consis-tency	pH	SAT (min)	MBRT (min)	PROTOZOA Mot.	Den.	TPC $_5$ x10 /ml	NIP. /UIP	LP. /UDP	Total BC $_8$ 10 /ml x
1.	101.0	17	45	2	Greenish brown	Aromatic	Slight viscous	6.7	5	8	+2	+2	3.33	0.21	3.44	7.2
2.	101.4	17	47	2	-do-	-do-	-do-	6.8	6	6	+3	+3	4.44	1.26	4.44	7.33
3.	102.0	20	50	2	Brownish green	Slight putrid	-do-	7.6	15	14	+2	+1	1.78	2.94	1.25	6.53
4.	101.0	16	45	3	-do-	Slight acid	-do-	6.7	4	7	+2	+2	4.33	0.92	3.16	7.73
5.	101.4	22	50	3	Brown	Aromatic	Watery	7.0	3	3	+3	+3	3.78	2.66	5.0	8.32
6.	101.2	17	52	3	-do-	-do-	Slight vicous	7.0	6	5	+2	+2	4.44	0.92	3.44	8.43
7.	100.8	16	54	2	-do-	-do-	-do-	6.9	4	5	+2	+2	4.67	0.96	4.57	9.49
8.	101.0	15	55	3	Green	-do-	-do-	7.0	5	3	+3	+3	4.33	0.37	3.44	10.43

Table 4.3 contd.
Group B

Case No.	Temp. °F	Resp. /min	Pulse /min	Rumen mot./ min	Colour	Odour	Consis-tency	pH	SAT (min)	MBRT (min)	PROTOZOA Mot.	PROTOZOA Den.	PROTOZOA TPC_5 x10^5 /ml	PROTOZOA NIP. /UIP	PROTOZOA LP. /UDP	Total BC_8 x 10^8 /ml
1.	101.4	16	50	2	Brownish green	Aromatic	Slight viscous	7.0	4	3	+2	+2	4.44	2.21	3.44	7.17
2.	101.0	18	52	3	Brown	-do-	-do-	6.8	4	3	+3	+3	2.00	0.92	4.44	7.44
3.	101.4	17	60	3	Greenish Brown	-do-	-do-	7.0	3	3	+2	+3	2.44	1.26	3.16	9.60
4.	101.4	20	60	2	Brown	-do-	-do-	7.1	4	3	+2	+2	3.78	2.66	3.52	9.33
5.	102.0	18	56	2	Light brown	-do-	-do	7.0	6	7	+2	+2	2.78	0.92	3.67	7.31
6.	101.0	17	55	2	Brownish green	Slight acid	-do-	6.7	3	4	+2	+3	3.00	0.96	3.92	8.57
7.	102.0	17	56	3	Brown	-do-	Watery	6.7	3	4	+2	+2	2.56	0.92	3.16	9.63
8.	101.2	18	52	3	Brownish green	-do-	-do-	6.8	4	3	+3	+3	3.11	0.96	4.44	9.07

TABLE No. 4.3 (contd)
Group C

Case No.	Temp. °F	Resp. /min	Pulse /min	Rumen mot./ min	Colour	Odour	Consis-tency	pH	SAT (min)	MBRT (min)	PROTOZOA Mot.	PROTOZOA Den.	PROTOZOA TPC $x10^5$ /ml	PROTOZOA NIP. /UIP	PROTOZOA LP. /UDP	Total BC 10^8 x /ml
1.	101.2	18	58	3	Green	Ammoniacal	Slight viscous	6.7	5	3	+3	+2	2.22	1.11	4.44	7.13
2.	101.0	20	60	2	-do-	Aromatic	-do-	7.0	3	3	+2	+3	3.0	0.37	3.12	7.2
3.	101.0	22	62	3	-do-	-do-	-do-	7.0	5	5	+3	+3	2.56	0.21	3.44	8.23
4.	101.4	22	63	3	Brown	-do-	-do-	6.9	4	3	+3	+3	3.78	0.18	3.69	8.0
5.	101.2	19	63	2	-do-	Ammoniacal	Watery	7.0	4	3	+2	+2	2.44	0.92	3.21	9.63
6.	102.0	16	60	3	-do-	Aromatic	Slight viscous	6.7	6	4	+4	+3	3.22	1.26	3.44	8.23
7.	101.0	17	55	2	-do-	-do-	Watery	7.0	5	4	+3	+2	2.22	1.11	2.52	9.2
8.	101.0	17	52	3	Greenish brown	Ammoniacal	Slight viscous	7.0	5	3	+3	+3	3.56	0.37	2.92	8.85

Temp. - Temperature.
Resp. - Respirations.
Mot. - Motility.
SAT. - Sedimentation activity test.
MBRT. - Methylene blue reduction time.
Den. - Density.
min. - minutes.

TPC. - Total protozoal count.
NIP. - Non iodophilic protozoa.
UIP. - Unit of iodophilic protozoa.
LP. - Live protozoa.
UDP. - Unit of dead protozoa.
BC. - Bacterial count.

Results And Discussion

RESULTS AND DISCUSSION

Anorexia is a common entity confronting the veterinarian and drawing the attention of the dairy farmers, since it directly reflects on the milk yield of the animals and the economy of the farmer. Hence it is imperative to diagnose such cases at an early stage and take prompt remedial measures and prevent economic losses.

5.1. DIAGNOSIS

5.1.1 *Clinical examination* -

The observations were made on 32 milch buffaloes which included four groups (A,B,C and D) of eight animals each. The animals of group D served as control. By obtaining proper history in a definite format, the cases of primary anorexia and secondary anorexia were detected and such cases were further subjected to detailed investigations.

It was noticed that the rumen motility was markedly decreased in all the cases and was ranging between one per 2.5 to 3 min. as compared to the control group where in it was 1 to 3 per minute. the procedure adopted in the present work was according to the outlines indicated by Dirksen (1983). Ruminal motility could be taken as a clinical index for diagnosis of rumen disorders (Boddie,1946; Blood *et al*

1983). The decrease in ruminal motility could be attributed to the digestive dysfunctions

5.1.2 *RUMEN FLUID*:

1. *Collection of rumen fluid*:

'Rumen Fluid Extraction Pump' was used for collecting rumen fluid samples.It obviated the necessity to puncture the rumen wall and enabled the collection of large quantity of rumen fluid with ease. Rumen fluid samples collected by this method were found to be sensitive to detect unfavourable changes related to digestive disturbances.Similar results were obtained by Raun and Burroughs.(1962) ,Gnanaprakasam (1979),Dirksen (1983) and Arle (1989).

2. *Observations on rumen fluid*:

a) Variation was found in colour,odour and consistency in clinical cases of anorexia. Such variations are often seen in acid or alkaline indigestion. (Dirksen, 1983 ; Blood *et al* 1983).

b) pH was found to vary between 6.0 to 7.0 and 7.1 to 8.5 indicating non-specific anorexia and alkaline indigestion respestively. In the control group,the pH was in the range of 6.0 to 7.1. In all 21 cases of alkaline indigestion and 3 cases of non-specific anorexia with pH range between 7.1 to

8.5 and 6.0 to 7.0 respectively .

c) Sedimentation activity test (SAT): An increase in SAT (4 to 19 min) was observed in all animals of group A,B and C. Statistical analysis of SAT revealed highly significant (P<0.01) increased in all the affected animals. The mean values were 11.75 ± 1.60, 9.38 ± 0.42 and 7.50 ± o.63 min. in groups A, B and C respectively.The increase could be due to variation in the rumen fluid pH which might have resulted into destruction of the ruminal microflora. An active microfloral population is necessary to produce gas that helps fibrous material to float. The present results are in accordance with the observations of Garry (1990).

d) Methylene blue reduction time (MBRT): There was an increase in MBRT in group A,B and C (3 to 20 min.)as compared to group D (3 to 5 min.). The mean values of MBRT were 11.38 ± 1.71, 6.75 ± 1.15 and 6.75 ± 0.82 min in grops A, B, and C respectively. Statistical analysis of MBRT revealed highly significant (P<0.01)increase indicating microbial inactivity of ruminal fluid. This is in correspondence with the observations of Garry (1990).

e) Protozoal motility: Motility of the protozoa was markedly decreased (+ 1 to + 2) in all the animals of groups A,B and C.

f) Protozoal density : Density of the protozoa was decreased markedly (+ 1 to + 2) in all the animals of group A,B and C.

g) Proportion of dead to live protozoa : A decrease in live protozoal proportion with an increase in dead protozoa was observed (0.52 to 6.69) per unit of dead protozoa in all anorectic animals. The mean values of live protozoa were 1.82 $\pm$ 0.63, 1.53 $\pm$ 0.76 and 1.85 $\pm$ 0.26/unit of dead protozoa in groups A, B and C respectively. Statistical analysis revealed highly significant ($P<0.01$) decrease in live protozoal proportion in all cases of anorexia. This decrease in live protozoal number could be attributed to variation in pH resulting into reduction in protozoal number. Similar observations were made by Dirksen (1983) and Arora (1983).

h) Iodophilic activity of protozoa : A decrease in iodophilic activity of protozoa with an increase in non-iodophilic protozoal proportion per unit of iodophilic protozoa were observed (0.86 to 6.43). The mean values of non iodophilic protozoa were 3.22 $\pm$ 0.92, 3.03 $\pm$ 0.68 and 1.81 $\pm$ 0.46 /unit of iodophilic protozoa in groups A, B and C respectively. Statistical analysis revealed significant ($P<0.05$) increase in non-iodophilic protozoa in group A where as highly significant increase ($P<0.01$) in groups B and C.

i) Total protozoal count (TPC) : A decreased in TPC was observed (0.77 to 4.88 x 10^5/ml of rumen fluid) in all anorectic animals. The mean values of total protozoa were 2.04 $\pm$ 0.41, 1.91 $\pm$ 0.33 and 1.84 $\pm$ 0.46 x 10^5/ml of rumen fluid in groups A, B and C respectively. Statistical analysis revealed significant ($P<0.05$) decrease in TPC in all cases of anorexia. This decrease in TPC could be attributed to pH variation resulting into destruction of protozoa. Similar findings were obtained by Dash *et al*, (1972) in their studies on acute indigestion in cattle.

j) Total bacterial count (TBC) : A decrease in TBC was observed 6.93 $\pm$ 0.27 x 10^8 /ml, 6.56 $\pm$ 0.11 x 10^8/ml and 6.69 $\pm$ 0.33 x 10^8 /ml of rumen fluid in all anorectic animals. Statistical analysis revealed highly significant ($P < 0.01$) decrease in total bacterial count in all cases of anorexia .

The variation in rumen fluid parameters in cases with pH 6.0 to 7.0 (non-specific anorexia) could probably be related to the feeding of poor quality feed without concentrates (Misra and Tripathy, 1963; Sopori and Prasad, 1987). In a high pH around neutrality or in the alkaline range, a putrid decomposition of the digesta develops, in the course of which toxic decomposition products are formed. pH in the range between 7.5 and 8.5 greatly reduces the number of protozoa (Dirksen , 1983). Statistical analysis for rumen fluid parameters before treatment are given in table 5.1.

5.2. HAEMATOLOGICAL AND BIOCHEMICAL STUDIES :-

5.2.1. HAEMATOLOGICAL STUDIES :-

The results of haemogram studies indicated that Hb (13.55 ± 0.66, 13.46 ± 0.14 and 13.10 ± 0.48 gm%) and PCV (40.88 ± 2.20, 44.00 ± 1.38 and 40.17 ± 0.40 %) in groups A, B and C respectively were found to be within the normal ranges as mentioned by Jain *et al*, (1986). These findings are in aggreement with the results obtained by Misra and Singh , (1974), though Dash *et al*, (1972) found haemoconcentration and increased PCV in cases of acute indigestion in cattle which might be due to severe dehydration. Differential leukocyte count revealed that there was neutrophilia and lymphocytopenia which were significant ($P< 0.05$) in group A and highly significant ($P<0.01$) in groups B and C whereas eosinophil, monocyte and basophil percentage were within the normal range. These observations are in accordance with the results obtained by Dash *et al*, (1972) and Hedaoo *et al*, (1982).

The neutrophilia could be attributed to the stress of digestive disorder in group A, B and C , due to which there will be release of corticosteroides which may cause neutrophilia and relative lymphocytopenia (Sastry, 1983).

5.2.2. BIOCHEMICAL STUDIES :-

Blood glucose levels were found to be increased significantly ($P<0.01$) in all cases of primary anorexia whereas in secondary anorexia due to ketosis it was found to be decreased (case no. 5 of group B (62.2 mg/dL) and case no. 1 and 3 of group C (48.6 and 48.0 mg/dL respectively.). Mean blood glucose levels were 70.48 $\pm$ 0.77, 72.53$\pm$ 1.11 and 75.77 $\pm$ 1.92 mg/dL in groups A, B and C respectively. The hyperglycaemia could be attributed to the stress of digestive disorder leading to increase release of adrenocorticoids which cause glycogenolysis and there by hyperglycaemia (Singh *et al*, 1989). The hypoglycaemia observed in 3 cases could be due to ketosis from which they were found to be suffering (Rothera's test on urine was positive).

Cholesterol and SGOT levels were found to be increased significantly ($P< 0.01$) in all cases of anorexia (primary and secondary). Mean cholesterol levels of 228.57 $\pm$ 5.50, 203.62 $\pm$ 7.55, 203.30 $\pm$ 6.97 mg/dL and SGOT levels of 60.04 $\pm$ 1.36, 58.33 $\pm$ 3.24 and 55.12 $\pm$ 2.80 I.U./L were observed in groups A, B and C respectively. Increased cholesterol levels may probably be due to it's decreased uptake by the damaged liver where cholesterol is metabolised to bile acids (Galhotra and Gupta, 1988). Increased SGOT could have resulted either by absorption of toxic metabolites from rumen in alkalosis (Cornelius and Kaneko, 1963) and hepatic necrosis (Dukes,

1970) or could be ascribed to hepatic insufficiency (Boyed *et al*, 1964).

The observation on blood glucose in the present study were in accordance with the findings of Sethuraman and Rathor (1979) and Hedaoo *et al*, (1982), Ahuja *et al*, (1988) and Singh and Kasarlikar (1988). Observations on cholesterol and SGOT were similar to the previous findings of Singh and Kasarlikar (1988) and Ahuja *et al*, (1988).

The statistical analysis for haematological and biochemical parameters before treatment are given in table 5.3.

5.3 CLINICAL RESPONSE TO TREATMENT :-

In the present study, cases of anorexia were treated with a probiotic bolus (group A), Rumenotoric bolus (group D) and herbal preparation (group C). The duration of treatment varied from 3 to 5 days. The statistical analysis for rumen fluid parameters after treatment are given in table 5.1 and 5.2, for haematological and biochemical parameters in table 5.3 and 5.4.

5.3.1. TREATMENT WITH PROBIOTIC BOLUS (GROUP A) :-

The cases of anorexia treated with a probiotic bolus showed improvement in their appetite by 2nd to 3rd day and it was completely restored by 5th day in 6 out of 7 animals (of primary anorexia) by 3rd day. Improvement was seen in rumen

motility (2 to 3 /min), SAT (3 to 6/min), MBRT (3 to 8/min), protozoal motility (+2 to +3), protozoal density (+2 to +3), total protozoa (3.33 to 4.67 x 10^5/ml of rumen fluid), non-iodophils (0.21 to 2.66 per unit of iodophilic protozoa), live protozoa (3.16 to 5.0 per unit of dead protozoa) and total bacteria (8.18 $\pm$ 0.45 x 10^8/ml of rumen fluid) in all treated animals. Case no. 3 does not show response with this therapy, since she was suffering from high temperature, metritis and mastitis.

Blood glucose (62.79 $\pm$ 0.46 mg/dL), cholesterol (180.80 $\pm$ 6.88 mg/dL) and SGOT (46.52 $\pm$ 4.03 I.U./L) were significantly ($P<0.01$) decreased. Still these values are higher than the controls (group D). However, paired 't' test revealed significant ($P<0.01$) decrease in their mean values when compared with pretreament values (70.48$\pm$ 0.77 mg/dL ,228.57$\pm$5.50mg/dL and 60.04$\pm$1.36I.U./L respectively) indicating their tendency to return to normal after treatment. Values for rumen fluid parameters, *viz.* pH (6.96$\pm$0.10), SAT (6.00$\pm$1.34min.), MBRT 6.38$\pm$1.25), total protozoa (4.19$\pm$0.18 x 10^5 /ml of rumen fluid), non-iodophilic protozoa (1.04$\pm$0.30 per unit iodophilic protozoa), live protozoa (3.93$\pm$0.27 per unit of dead protozoa), total bacteria (8.18$\pm$0.45 x 10^8/ml of rumen fluid) indicating their tendency to return to normal after treatment. Hb and PCV were found to be within the normal range even before treatment (13.55$\pm$0.66 gm% and 40.88$\pm$2.20% respectively). Therefore they

did not differ significantly with values after treatment (13.68±0.61gm% and 40.75±0.50% respectively). Improvement in neutrophilia, lymphocytopenia was seen in all the cases of this group. Improvement was also seen in colour, odour, consistency and pH of rumen fluid on 3rd to 5th day of treatment in all the animals except case No.3.

Probiotic (Bioboost bolus) used in this study contained live yeast culture, amino-acids, culture of live *Lactobacillus sporogenes* and liver extract which might have improved digestibility of the nutrients in the feed as suggested by Wu (1987) and also improved the rumen pH (Newman and Dawson,1987). As a result of this rumen microbes might have been activated leading to improved rumen motility and thereby improvement in the appetite. This observation correspond to the statement of Blood and Radostits, (1989) that rumen microflora and rumen motility are interdependent on each other. Thus improvement in rumen microflora leads to improvement in rumen motility.

Case No.3 showed high temperature (105^{0}F) when presented for treatment and was suffering from metritis and mastitis. Its rumen liquor parameters were : pH (8.0), brown colour, slight putrid odour and slight viscous consistency, increase in SAT (19 min) and MBRT (20 min), decrease protozoal motility and density (+1), decreased total protozoal count (1.66 x 10^{5}/ml of rumen fluid, Non-iodophils (2.66/unit of iodophilic

protozoa), live protozoa (0.69/unit of dead protozoa) and total bacteria (6.83 x 10^{8}/ml of rumen fluid), decreased rumen motility (1/3 min) This animal was being treated simultaneously for the primary conditions (metritis and mastitis) along with therapy for secondary anorexia for a period of 8 days. Inspite of the combined therapy the animal did not respond and continued to be anorectic as evidenced by its rumen liquor parameters at the end of 8 days treatment (brownish green colour, slight putrid odour, slight viscous consistency, pH (7.6), SAT (15 min), MBRT (14 min) protozoal motility (+2), protozoal density (+1), Total protozoa (1.78 x 10^{5}/ml of rumen fluid), non-iodophilis (2.94/unit of iodophilic protozoa, live protozoa (1.25/unit of dead protozoa) and total bacteria (6.53 x 10^{8}/ml of rumen fluid). The results of the present study showed that probiotic cured 6 out of 7 cases of primary anorexia with three days giving cure rate of 85.71% where as remaining case required 5 days treatment for recovery.

5.3.2 : TREATMENT WITH RUMENOTORIC BOLUS (Group B).

The anorectic animals treated with rumenotoric bolus showed improvement in their appetite by the third day of treatment in all the seven cases of primary anorexia. However, in case no. 5 required treatment for 5 days as it was suffering from ketosis. Simultaneous therapy with propylene glycol was resorted in this case to treat the ketosis.

There was improvement in pH (6.7 to 7.1), rumen motility (2 to 3/min), protozoal motility and density (+2 to +3) SAT (3 to 6 min), MBRT (3 to 7 min), protozoal density (+2 to +3), total protozoal count (2 to 4.44 x 10^5/ml of rumen fluid), decrease in non-iodophilic protozoa in (0.92 to 2.66/unit of iodophilic protozoa), increase in live protozoal proportion (3.16 to 4.44/unit of dead protozoa), Total bacteria (7.17 to 9.63 x 10^8/ml of rumen fluid).

Similarly, there was an appreciable improvement in blood glucose level(62.75 $\pm$ 0.94 mg/dL), cholesterol (162.50 $\pm$ 8.49 mg/dL) and SGOT (49.32 $\pm$ 3.15 I.U./L) although these values were still found to be significantly ($P<0.01$) higher than the control values of group D (61.93 $\pm$ 0.25 mg/dL, 96.59 $\pm$ 4.19 mg/dL and 30.05 $\pm$ 0.69 I.U./L respectively). However they showed a tendency to return to normal after treatment, which was evident from the highly significant ($P < 0.01$) difference from the pretreatment values (72.53 $\pm$ 1.11 mg/dL, 203.62 $\pm$ 7.55 mg/dL and 58.52 $\pm$ 3.24 I.U./L respectively). Among the haematological values, there was improvement in the neutrophilia, lymphocytopenia after treatment. All other values (Hb and PCV) were found to be within the normal range even before treatment (13.46 $\pm$ 0.14 gm% and 44.00 $\pm$ 1.38% respectively) and hence, they did not differ significantly with the values after treatment (13.51 $\pm$ 0.10 gm%, 43.14 $\pm$ 0.99% respectively).

Similarly there was considerable improvement in the colour, odour, consistency and pH of the rumen fluid on third day of the treatment in all animals except case no. 5.

Case No. 5 showed alkaline indigestion when presented for treatment, as evidenced by an increase in pH (8.0), light brown in colour, ammoniacal odour, slight viscous in consistency of rumen fluid, increased SAT (10 min), MBRT (14 min) decrease protozoal motility and density (+1), decreased total protozoal count (1.11 x 10^5/ml of rumen fluid), non-iodophilic protozoal proportion (1.5/unit of iodophilic protozoa), live protozoa (0.4/unit of dead protozoa) and total bacterial count (6.3 x 10^8/ml of rumen fluid), decreased rumen motility (1/3 min).

Gentian and ginger present in the rumenotoric bolus might have been responsible for improving the rumen motility (Fraser *et al* (1986). Other ingredients in the bolus such as methionine, cobalt sulphate, copper sulphate, vitamin B_1, nicotinamide, dried yeast and dextrose might have helped to restore the microbial activity as they are known to be acting as substrates for the growth of rumen microbes (Booth and McDonald, 1988). Increased rumen motility and microbial activity must have lead to the restoration of appetite in treated animals (Fraser *et al* 1986).

The above results showed that the rumenotoric bolus gave a cure rate of 100% in treated cases of primary anorexia within three days.

5.3.3 : TREATMENT WITH HERBAL PREPARATION. (Group C).

The anorectic animals treated with the herbal preparation (rumenotoric) showed improvement in their appetite by third day to fifth day of treatment. By third day the appetite was restored completely in five out of six cases of primary anorexia. There were two cases of secondary anorexia - case no.1 and 3, which required simultaneous treatment for the primary conditions (ketosis). They showed response by third day and seventh day respectively.

There was improvement in rumen motility (2 to 3/min) pH (6.7 to 7.0), SAT (3 to 6 min), MBRT (3 to 5 min) protozoal motility (+2 to +4),total protozoal count(2.22 to 3.78 $x10^5$/ml of rumen fluid), non-iodophilic protozoa (0.18 to 1.26/unit of iodophilic protozoa), live protozoal proportion (2.52 to 4.44/unit of dead protozoa), total bacterial count (7.13 to 9.63) into 10^8/ml of the rumen fluid). Similarly there was appreciable improvement in the blood glucose levels (64.36 $\pm$ 0.55 mg/dL), Cholesterol (156.25 $\pm$ 6.59 mg/dL) and SGOT (42.87 $\pm$ 2.39 I.U/L) although these values were still found to be higher than the control values of group D (61.93 $\pm$ 0.25 ml/dL, 96.59 $\pm$ 4.19 mg/dL and 30.05 $\pm$ 0.69 I.U./L respectively). However they showed a tendency to return to

normal after treatment, which was evident from the highly significant ($P<0.01$) difference from the pretreatment values (75.77 ± 1.92 mg/dL, 203.30 ± 6.97 mg/dL and SGOT 55.12 ± 2.80 I.U./L respectively). Among the haematological values there was improvement in the neutrophilia, lyphocytopenia after treatment. All other values (Hb and PCV) were found to be within the normal range even before treatment (13.10 ± 0.48 gm%, 40.17 ± 0.40 %) and hence, they did not differ significantly with values after treatment (13.20 ± 0.41 gm% and 39.33 ± 0.88 % respectively). Similarly there was considerable improvement in the colour, odour, consistency and pH o the rumen fluid on third to fifth day in all animals except case no. 3.

The results of the present study showed that the herbal preparation (Rumbion Bolus) cured five out six cases of primary anorexia within three days giving cure rate of 83.33% where as remaining case required five days of treatment for recovery.

Rumbion contains herbal ingredients like *Picrorhiza kurrooa*, *Zingiber officinale*, *Andrographis paniculata*, *Trachyspermum ammi*, *Piper nigrum* which are reported to be useful in digestive tract dysfunctions. According to Chopra (1958) *Picrorhiza kurrooa* is a bitter tonic almost as efficacious as gentian. It acts as antiperiodic and cholagogue. *Zingiber officinale* is a carminative and an aromatic stimulant. It is

frequently given with purgatives to reduce griping. *Andrographis paniculata* also acts as a bitter. *Trachyspermum ammi* is a powerful antiseptic and aromatic carminative, tonic and antispasmodic. Jain (1968) reported that, leaves and fruits of *Piper nigrum* have an antibacterial activity.

TABLE No : 5.1.

ANALYSIS OF VARIANCE FOR RUMEN FLUID PARAMETERS OF BUFFALOES SUFFERING FROM ANOREXIA (GROUPS A, B AND C) BEFORE AND AFTER TREATMENT

Source	D.F	MEAN SQUARES						
		pH	SAT	MBRT	Total protozoa	Non iodophilic protozoa	Live protozoa	Total bacteria
BEFORE TREATMENT.								
Between groups	2	0.72^{NS}	36.29^{*}	57.05^{*}	0.08^{NS}	4.69^{NS}	0.10^{NS}	0.28^{NS}
Error	21	0.24	8.35	13.09	1.29	4.02	2.79	0.51
Total	23							
Comparison of Means.								
Groups								
A		$8.06 \pm 0.11^{**}$	$11.75 \pm 1.60^{**}$	$11.38 \pm 1.71^{**}$	$2.04 \pm 0.41^{*}$	$3.22 \pm 0.92^{*}$	$1.82 \pm 0.63^{*}$	$6.93 \pm 0.27^{**}$
B		$7.46 \pm 0.22^{*}$	$9.38 \pm 0.42^{**}$	$6.75 \pm 1.15^{**}$	$1.91 \pm 0.33^{*}$	$3.03 \pm 0.68^{*}$	$1.53 \pm 0.76^{*}$	$6.56 \pm 0.11^{**}$
C		$7.79 \pm 0.16^{**}$	$7.50 \pm 0.63^{**}$	$6.75 \pm 0.82^{**}$	$1.84 \pm 0.46^{*}$	$1.81 \pm 0.46^{*}$	$1.85 \pm 0.26^{**}$	$6.69 \pm 0.33^{*}$
AFTER TREATMENT.								
Between groups	2	0.01^{NS}	9.29^{NS}	18.02^{*}	2.41^{*}	1.05^{NS}	0.29^{NS}	0.22^{NS}
Error	21	0.04	5.37	5.02	0.63	0.56	0.64	1.19
Total	23							
Comparison of Means.								
Groups								
A		$6.98 \pm 0.10^{**}$	$6.00 \pm 1.34^{**}$	$6.38 \pm 1.25^{**}$	$3.69 \pm 0.34^{*}$	$1.28 \pm 0.35^{*}$	$3.59 \pm 0.34^{*}$	$8.18 \pm 0.45^{**}$
B		$6.89 \pm 0.10^{*}$	$3.88 \pm 0.35^{**}$	$3.75 \pm 0.49^{**}$	$3.01 \pm 0.28^{*}$	$1.35 \pm 0.24^{*}$	$3.72 \pm 0.18^{*}$	$8.51 \pm 0.38^{**}$
C		$6.91 \pm 0.48^{**}$	$4.63 \pm 0.32^{**}$	$3.50 \pm 0.27^{**}$	$2.88 \pm 0.21^{*}$	$0.69 \pm 0.16^{*}$	$3.35 \pm 0.20^{**}$	$8.31 \pm 0.32^{*}$

Note :

NS - Non significant

( ) - indicate D.F for that parameter.

D.F - Degrees of Freecom.

* - Significant at 5% level ($P < 0.05$) For variance ratio,

** - Significant at 1% level ($P < 0.01$) For variance ratio

TABLE No. 5.2

COMPARISON OF MEANS OF RUMEN FLUID PARAMETERS OF BUFFALOES SUFFERING FROM ANOREXIA (GROUPS A, B and C) BEFORE AND AFTER TREATMENT AND APPARENTLY HEALTHY ANIMALS (GROUP D).

Group	PARAMETERS	MEANS				't' VALUES	n-1
		BEFORE TREATMENT		AFTER TREATMENT			
A	pH	8.06	± 0.11	6.96	± 0.10	6.813**	7
	SAT	11.75	± 1.60	6.00	± 1.34	6.243**	7
	MBRT	11.38	± 1.71	6.38	± 1.25	3.669**	7
	Total protozoa x 10^5/ml	2.04	± 0.41	3.89	± 0.34	3.217*	7
	Non iodophilic protozoa	3.22	± 0.92	1.28	± 0.35	2.597*	7
	Live protozoa	1.82	± 0.63	3.59	± 0.34	2.958*	7
	Total bacteria x 10^8/ml	6.93	± 0.27	8.18	± 0.45	4.097**	7
B	pH	7.46	± 0.22	6.89	± 0.10	2.595*	7
	SAT	9.38	± 0.42	3.88	± 0.35	14.552**	7
	MBRT	6.75	± 1.15	3.75	± 0.49	4.099**	7
	Total protozoa x 10^5/ml	1.91	± 0.33	3.01	± 0.28	2.468*	7
	Non iodophilic protozoa	3.03	± 0.68	1.35	± 0.24	3.377*	7
	Live protozoa	1.53	± 0.76	3.72	± 0.18	3.209*	7
	Total bacteria x 10^8/ml	6.56	± 0.11	8.51	± 0.38	6.167**	7

TABLE No. 5.2 (Contd.)

Group	PARAMETERS	MEANS BEFORE TREATMENT	MEANS AFTER TREATMENT	't' VALUES	n-1
	pH	7.79 ± 0.16	6.91 ± 0.48	4.568**	7
	SAT	7.50 ± 0.63	4.63 ± 0.32	7.222**	7
	MBRT	6.75 ± 0.82	3.50 ± 0.27	5.017**	7
C					
	Total protozoa x 10^5/ml	1.84 ± 0.46	2.88 ± 0.21	2.690*	7
	Non iodophilic protozoa	1.81 ± 0.46	0.69 ± 0.16	3.417*	7
	Live protozoa	1.85 ± 0.26	3.35 ± 0.20	4.138**	7
	Total bacteria x 10^8/ml	6.69 ± 0.33	8.31 ± 0.32	3.218*	7
	pH	6.94 ± 0.05	- - -		7
	SAT	5.50 ± 0.19	- - -		7
	MBRT	4.13 ± 0.30	- - -		7
D					
	Total protozoa x 10^5/ml	3.82 ± 0.46	- - -		7
	Non iodophilic protozoa	0.78 ± 0.15	- - -		7
	Live protozoa	6.19 ± 1.09	- - -		7
	Total bacteria x 10^8/ml	9.91 ± 0.50	- - -		7

NOTE: * -- Significant at 5% level ($P < 0.05$)
** -- Significant at 1% level ($P < 0.01$)
n-1-- Degrees of Freedom.

TABLE No. : 5.3

ANALYSIS OF VARIANCE FOR HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS OF BUFFALOES SUFFERING FROM ANOREXIA (GROUPS A, B AND C) BEFORE AND AFTER TREATMENT.

		MEAN SQUARES				
Source	D.F	Neutrophil	Lymphocyte	Blood glucose	Cholesterol	SGOT.
BEFORE TREATMENT.						
Between groups	2	9.02^{NS}	31.01^{NS}	48.07^{*}	(2) 1681.06^{*}	(2) 101.80^{NS}
Error	18	30.92	23.40	10.49	(21) 362.15	(21) 29.08
Total	20				(23)	(23)
Comparison of Means.						
Groups						
A		$51.63 \pm 2.94^{*}$	$42.0 \pm 2.42^{**}$	$70.48 \pm 0.77^{**}$	$228.57 \pm 5.50^{**}$	$60.04 \pm 1.36^{**}$
B		$50.71 \pm 1.19^{**}$	$45.57 \pm 1.27^{**}$	$72.53 \pm 1.11^{**}$	$203.62 \pm 7.55^{**}$	$56.33 \pm 3.24^{**}$
C		$49.33 \pm 0.67^{**}$	$45.50 \pm 0.92^{**}$	$75.77 \pm 1.92^{**}$	$203.30 \pm 6.97^{**}$	$55.12 \pm 2.80^{**}$
AFTER TREATMENT.						
Between groups	2	9.91^{NS}	4.73^{NS}	5.41^{NS}	(2) 1256.78^{NS}	(2) 83.63^{NS}
Error	18	3.30	6.20	2.92	(21) 434.69	(21) 84.98
Total	20				(23)	(23)
Comparison of Means.						
Groups						
A		$44.13 \pm 0.52^{*}$	$47.75 \pm 1.08^{**}$	$82.79 \pm 0.46^{**}$	$180.80 \pm 6.88^{**}$	$46.52 \pm 4.03^{**}$
B		$46.43 \pm 0.84^{**}$	$46.86 \pm 0.88^{**}$	$82.75 \pm 0.94^{**}$	$162.50 \pm 8.49^{**}$	$49.32 \pm 3.15^{**}$
C		$45.17 \pm 0.70^{**}$	$49.33 \pm 0.67^{**}$	$84.36 \pm 0.55^{**}$	$156.25 \pm 6.59^{**}$	$42.87 \pm 2.39^{**}$

Note :

NS - Non significant

( ) - indicate D.F for that parameter.

D.F - Degrees of Freedom.

* - Significant at 5% level ($P < 0.05$) For variance ratio,

** - Significant at 1% level ($P < 0.01$) For variance ratio

TABLE No. : 5.3

ANALYSIS OF VARIANCE FOR HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS OF BUFFALOES SUFFERING FROM ANOREXIA (GROUPS A, B AND C) BEFORE AND AFTER TREATMENT.

		MEAN SQUARES				
Source	D.F	Neutrophil	Lymphocyte	Blood glucose	Cholesterol	SGOT.
BEFORE TREATMENT.						
Between groups	2	9.02^{NS}	31.01^{NS}	48.07^{*}	(2) 1681.06^{*}	(2) 101.80^{NS}
Error	18	30.92	23.40	10.49	(21) 362.15	(21) 29.08
Total	20				(23)	(23)
Comparison of Means.						
Groups						
A		$51.63 \pm 2.94^{*}$	$42.0 \pm 2.42^{**}$	$70.48 \pm 0.77^{**}$	$228.57 \pm 5.50^{**}$	$60.04 \pm 1.36^{**}$
B		$50.71 \pm 1.19^{**}$	$45.57 \pm 1.27^{**}$	$72.53 \pm 1.11^{**}$	$203.62 \pm 7.55^{**}$	$58.33 \pm 3.24^{**}$
C		$49.33 \pm 0.67^{**}$	$45.50 \pm 0.92^{**}$	$75.77 \pm 1.92^{**}$	$203.30 \pm 6.97^{**}$	$55.12 \pm 2.80^{**}$
AFTER TREATMENT.						
Between groups	2	9.91^{NS}	4.73^{NS}	5.41^{NS}	(2) 1256.76^{NS}	(2) 83.63^{NS}
Error	18	3.30	6.20	2.92	(21) 434.69	(21) 84.98
Total	20				(23)	(23)
Comparison of Means.						
Groups						
A		$44.13 \pm 0.52^{*}$	$47.75 \pm 1.08^{**}$	$62.79 \pm 0.46^{**}$	$180.80 \pm 6.88^{**}$	$46.52 \pm 4.03^{**}$
B		$46.43 \pm 0.84^{**}$	$48.66 \pm 0.88^{**}$	$62.75 \pm 0.94^{**}$	$162.50 \pm 0.49^{**}$	$49.32 \pm 3.15^{**}$
C		$45.17 \pm 0.70^{**}$	$49.33 \pm 0.67^{**}$	$64.36 \pm 0.55^{**}$	$156.25 \pm 6.59^{**}$	$42.87 \pm 2.39^{**}$

Note :

NS - Non significant

( ) - indicate D.F for that parameter.

D.F - Degrees of Freedom.

* - Significant at 5% level ($P < 0.05$) For variance ratio,

** - Significant at 1% level ($P < 0.01$) For variance ratio

TABLE No. 5.4 :

COMPARISON OF MEANS OF HAEMATOLOGICAL AND BIOCHEMICAL VALUES OF BUFFALOES SUFFERING FROM ANOREXIA (GROUPS A, B and C) BEFORE AND AFTER TREATMENT AND APPARENTLY HEALTHY ANIMALS (GROUP D).

Group	PARAMETERS	MEANS						't' VALUES	n-1
		BEFORE TREATMENT			AFTER TREATMENT				
A	Hb (gm%)	13.55	±	0.66	13.68	±	0.61	1.930^{NS}	7
	PCV (%)	40.88	±	2.20	40.75	±	2.00	0.424^{NS}	7
	TRBCs(10^6/cmm)	5.04	±	0.72	4.99	±	0.50	0.169^{NS}	7
	TWBCs(10^3/cmm)	7.62	±	0.62	7.21	±	0.47	2.094^{*}	7
	Neutrophil (%)	51.63	±	2.94	44.13	±	0.52	2.393^{*}	7
	Lymphocyte (%)	42.00	±	2.42	47.75	±	1.08	4.004^{**}	7
	Eosinophil (%)	4.25	±	0.56	3.63	±	0.18	1.174^{NS}	7
	Monocyte (%)	1.75	±	0.49	2.75	±	0.25	1.528^{NS}	7
	Basophil (%)	0.38	±	0.18	0.50	±	0.19	0.424^{NS}	7
	Blood glucose (mg/dL)	70.48	±	0.77	62.79	±	0.46	7.792^{**}	7
	Cholesterol (mg/dL)	228.57	±	5.50	180.80	±	6.66	10.499^{**}	7
	SGOT (I.U./L)	60.04	±	1.36	46.52	±	4.03	5.803^{**}	7
B	Hb (gm%)	13.46	±	0.14	13.51	±	0.10	0.795^{NS}	7
	PCV (%)	44.00	±	1.38	43.14	±	0.99	1.867^{NS}	7
	TRBCs(10^6/cmm)	6.69	±	0.56	6.34	±	0.44	1.939^{NS}	7
	TWBCs(10^3/cmm)	6.31	±	0.20	6.29	±	0.19	1.000^{NS}	7
	Neutrophil(%)	50.71	±	1.19	46.43	±	0.84	6.159^{**}	7
	Lymphocyte(%)	45.57	±	1.27	48.86	±	0.88	4.073^{**}	7
	Eosinophil(%)	2.71	±	0.42	3.43	±	0.30	1.987^{NS}	7
	Monocyte (%)	0.71	±	0.29	1.43	±	0.20	1.698^{NS}	7
	Basophil (%)	0.29	±	0.18	0.57	±	0.20	1.000^{NS}	7
	Blood glucose(mg/dL)	72.53	±	1.11	62.75	±	0.94	9.494^{**}	6
	Cholesterol (mg/dL)	203.62	±	7.55	162.50	±	8.49	12.324^{**}	7
	SGOT (I.U./L)	58.33	±	3.24	49.24	+	3.15	6.355^{**}	7

TABLE No. 5.4 (Contd.)

Group	PARAMETERS	MEANS			't' VALUES	n-1
		BEFORE TREATMENT	AFTER TREATMENT			
C	Hb (gm%)	13.10 ± 0.48	13.20 ± 0.41		1.464^{NS}	6
	PCV (%)	40.17 ± 0.40	39.33 ± 0.66		1.166^{NS}	6
	TRBCs(10^6/cmm)	5.79 ± 0.47	5.73 ± 0.36		0.587^{NS}	6
	TWBCs(10^3/cmm)	8.69 ± 0.64	8.53 ± 0.63		1.890^{NS}	6
	Neutrophil (%)	49.33 ± 0.67	45.17 ± 0.70		4.406^{**}	6
	Lymphocyte (%)	45.50 ± 0.92	49.33 ± 0.67		4.394^{**}	6
	Eosinophil (%)	3.67 ± 0.33	3.17 ± 0.31		1.464^{NS}	6
	Monocyte (%)	1.50 ± 0.50	1.67 ± 0.42		0.277^{NS}	6
	Basophil (%)	0.17 ± 0.17	0.67 ± 0.21		2.236	6
	Blood glucose (mg/dL)	75.77 ± 1.92	64.36 ± 0.55		6.825^{**}	6
	Cholesterol (mg/dL)	203.30 ± 6.97	156.25 ± 6.59		8.634^{**}	7
	SGOT (I.U./L)	55.12 ± 2.80	42.87 ± 2.39		7.128^{**}	7
D	Hb (gm%)	11.58 ± 0.24	-	-	-	7
	PCV (%)	37.25 ± 1.57	-	-	-	7
	TRBCs(10^6/cmm)	5.91 ± 0.11	-	-	-	7
	TWBCs(10^3/cmm)	9.01 ± 0.56	-	-	-	7
	Neutrophil (%)	44.75 ± 0.41	-	-	-	7
	Lymphocyte (%)	49.63 ± 0.68	-	-	-	7
	Eosinophil (%)	3.63 ± 0.32	-	-	-	7
	Monocyte (%)	1.63 ± 0.18	-	-	-	7
	Basophil (%)	0.38 ± 0.18	-	-	-	7
	Blood glucose (mg/dL)	61.93 ± 0.25	-	-	-	7
	Cholesterol (mg/dL)	96.59 ± 4.19	-	-	-	7
	SGOT (I.U./L)	30.05 ± 0.69	-	-	-	7

NOTE: * -- Significant at 5% level (P < 0 .05)
** -- Significant at 1% level (P < 0 .01)
n-1-- Degrees of Freedom.

Summary And Conclusions

SUMMARY AND CONCLUSIONS

The present study was undertaken in view of the high incidence of Bovine rumen dysfunctions and the misleading diagnostic and therapeutic approaches adopted in the field. A total of 32 milch buffaloes were taken up for this study of which 24 were detected to be cases of anorexia (20 cases of primary anorexia and 4 cases of secondary anorexia) and remaining eight were apparently healthy animals, which served as controls (group D). The cases of anorexia were divided into three groups A,B and C comprising of eight animals in each group. These were further subjected to detailed investigations. Individual case history regarding feeding pattern was obtaining from the owners. Rumen motility was recorded.

Rumen fluid samples were collected by "Rumen Fluid Extraction pump" and studied for various parameters before and after treatment. Haematological studies (haemoglobin and packed cell volume and differential leukocyte count and biochemical changes (blood glucose, cholesterol and SGOT) were carried out both before and after treatment. All the above parameters were also studied in apparently healthy animals of group D.Animal in group A were treated with a probiotic (*Bioboost* bolus), group B with a rumenotoric (Floratone bolus) and

group C with a herbal preparation (Rumbion bolus).
Individual case history from the owners showed that change in feed, feeding poor quality feed were related to digestive disturbances in these animals. Rumen motility was found to be decreased in all cases of anorexia. Suction strainer technique making use of " Rumen Fluid extraction pump" was found to be handy, satisfactory and efficient method for collecting the rumen fluid samples in affected and healthy buffaloes when secured properly.

Variation was found in colour, odour and consistency of rumen fluid in clinical cases of anorexia. pH was found to vary indicating alkaline indigestion and non-specific anorexia. Sedimentation activity test (SAT), methylene blue reduction time (MBRT) were found to be increased significantly in both types of anorexia indicating reduced activity or reduced total count of rumen microflora and fauna as a result of the adverse effects of pH changes or of toxic metabolites in the rumen.

Haematological studies revealed non-significant variation in Hb and PCV in groups A, B and C as compared to group D. Differential leukocyte count showed significant neutrophilia and lymphocytopenia. Blood glucose, cholesterol and SGOT levels prior to treatment showed highly significant increase in primary anorexia whereas in secondary anorexia with ketosis blood sugar level was lower.

The results of treatment trials showed that the rumen motility improved in group A, B and C. Rumen fluid parameters showed excellent improvement (100%) in primary anorexia cases to treatment with rumenotoric bolus (Floratone bolus) followed by probiotic (Bioboost bolus) (85.71%) and herbal preparation (Rumbion bolus), (83.33%). Although neutrophilia and lymphocytopenia were evident even after treatment in all groups, they differed significantly with the pre-treatment values indicating that there was a tendency to return to normal. Statistical analysis of blood glucose, cholesterol and SGOT levels within groups, before and after treatment showed significant decrease indicating their tendency to return to normal.

From the present study the following conclusions were drawn:

1) Rumen fluid parameters like pH, SAT, MBRT were simple and sensitive tests to detect the cases of digestive dysfunctions in early stages. Protozoal motility, density, protozoal count, live and dead protozoal, iodophilic activity of protozoa, bacterial count were essential to detect the severity of dysfunction. All these parameters were found to be helpful in deciding the proper line of treatment and also to know the efficacy of treatment.

2) Haematological studies such as Hb and PCV were found to be of no diagnostic value although DLC, blood glucose,

cholesterol and SGOT proved to be helpful in diagnosis alongwith the rumen fluid examination in cases of anorexia.

3) In treating cases of primary anorexia in milch buffaloes the following were found to effective in their descending order :

a) Rumenotoric - Floratone bolus (100%)

b) Probiotic - Bioboost bolus (85.71%)

c) Herbal preparation - Rumbion bolus (83.33%)

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Appendices

APPENDIX - I

DETAILS OF BUFFALOES SUFFERING FROM ANOREXIA (GROUPS A, B AND C) AND APPARENTLY HEALTHY BUFFALOES (GROUP D) AND TREATMENT GIVEN TO THEM.

GROUP A — BIOBOOST BOLUS

No.	Farm	Breed	Age (yrs)	Treatment	Days to resume appetite: Partial	Days to resume appetite: Complete	Overall recovery %
(1)	Ramesh, Thane	Murrah	8	2BID for 5 days	3	5	
(2)	Unit No.22 Aarey Milk Colony	- do -	10	2BID for 3 days	2	3	
(3)	**Rajbali, Thane	- do -	7	2BID for 5 days	3	6*	
(4)	Jwala. Chembur	- do -	8	2BID for 3 days	2	3	85.71
(5)	Haribhai, Mulund.	- do -	8	2BID for 3 days	2	3	
(6)	Rajbali, Thane	- do -	9	2BID for 3 days	2	3	
(7)	Haribhai, Mulund.	- do -	8	2BID for 3 days	2	3	
(8)	Motibhai, Malad.	- do -	8	2BID for 3 days	2	3	

* - No improvement by 8 days therapy.

GROUP B — FLORATONE BOLUS

No.	Farm	Breed	Age (yrs)	Treatment	Days to resume appetite: Partial	Days to resume appetite: Complete	Overall recovery %
(1)	Unit No.22 Aarey Milk Colony.	Murrah	11	4BID for 3 days	2	3	
(2)	Jwala, Chembur.	- do -	8	4BID for 3 days	2	3	
(3)	Haribhai, Mulund.	- do -	8	2BID for 3 days	2	3	
(4)	Rajbali, Thane.	- do -	7	4BID for 3 days	2	3	100
(5)	**Birbal, Thane.	- do -	8	4BID for 5 days	3	5~	
(6)	Haribhai, Mulund.	- do -	9	4BID for 3 days	2	3	
(7)	Rajbali, Thane.	- do -	8	4BID for 3 days	2	3	
(8)	Larmedith	- do -	10	4BID for 3 days	2	3	

~ - No improvement in ketosis.

APPENDIX-I (Contd)

GROUP C. RUMBION BOLUS

No.	Farm	Breed	Age (yrs)	Treatment	Days to resume appetite		Overall recovery %
					Partial	Complete	
(1)	**Rajaram, Thane.	Murrah	8	2BID for 5 days	3	3	
(2)	Mehta, Thane.	- do -	8	2BID for 3 days	2	3	
(3)	**Pannalal,Digha.	- do -	8	2BID for 7 days	3	7	
(4)	Munnar, Thane.	- do -	9	2BID for 3 days	2	5	83.33
(5)	Vishnu B., Thane.	- do -	11	- do -	2	3	
(6)	Haribhai, Mulund.	- do -	8	- do -	2	3	
(7)	Haribhai, Mulund.	- do -	9	- do -	2	3	
(8)	Haribhai, Mulund.	- do -	8	- do -	2	3	

** indicating the case of secondary anorexia

GROUP D. CONTROL

No.	Tag No.	Farm	Breed	Age (yrs)	Treatment	Days to resume appetite		Overall recovery %
						Partial	Complete	
(1)	4800	Aarey Milk	Murrah	9	-	-	-	
(2)	4862	colony	- do -	10	-	-	-	
(3)	4831	- do -	- do -	9	-	-	-	
(4)	4794	- do -	- do -	9	-	-	-	
(5)	4760	- do -	- do -	11	-	-	-	
(6)	4757	- do -	- do -	10	-	-	-	
(7)	4748	- do -	- do -	10	-	-	-	
(8)	4742	- do -	- do -	9	-	-	-	

APPENDIX -II

PROFORMA OF RECORDING THE CASES OF ANOREXIA IN MILCH BUFFALOES

Name of the owner : _____ Case No. : _____

Address : _____ Date : _____

Age : _____ Sex : _____ Breed : _____

History

-Nature of the feed given by the owner : _____

-Any change effected in feeding schedule : Yes/No, if yes specify

-Symptom before onset of anorexia, if any : _____

CLINICAL EXAMINATION

Symptoms : _____

	Before treatment	After treatment
Temperature(^{O}F)	--	--
Resp.rate/min	--	--
Pulse/min	--	--
Mucus membranes a)Oral	--	--
b)Conjunctival	--	--
Any abnormality in mouth	--	--
External palpation of left flank	--	--
Rumination	--	--
Rumen motility	--	--
Other sign/findings if any	--	--

RUMEN FLUID EXAMINATION

Colour	--	--
Odour	--	--
Consistency	--	--
pH	--	--
SAT (min)	--	--

MBRT (min)	--	--
Rumen protozoa		
a.Motility	--	--
b.Density	--	--
c.Proportion of dead to live protozoa	--	--
d.Iodophilic activity of protozoa	--	--
e.Total protozoa	--	--
Rumen bacteria-total bacterial count	--	--

HAEMATOLOGICAL AND BIOCHEMICAL OBSERVATIONS

Haemoglobin (gm/100ml)	--	--
Packed cell volume (%)	--	--
Neutrophil (%)	--	--
Lymphocyte (%)	--	--
Eosinophil (%)	--	--
Monocyte (%)	--	-
Basophil (%)	--	-
Blood glucose (mg/dL)	--	--
Cholesterol (mg/dL)	--	--
SGOT (I.U/L	--	--

URINALYSIS

pH	--	--
Rothera's test	--	--
Sulkowitch test	--	--

DIAGNOSIS : _____

TREATMENT : _____

RESULT : _____

CERTIFICATE

The viva-voce examination of Mr. RAJIV VASANTRAO GAIKWAD was conducted on 8th June 1991 and the necessary correction/ modifications suggested by the External examiner and Advisory committee have been duly carried out and the thesis is submitted in the bound form for onward transmission.

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