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CLINICAL AND BIOCHEMICAL INVESTIGATIONS ON PUTREFACTION OF THE RETICULORUMINAL CONTENTS IN SHEEP

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SUMMARY

Ten 1.5-2 year old, native ewes were used in the present study. The disease was induced by feeding the ewes on deteriorated cabbage & turnip and straw pedding previously treated with ammonia. The diseased sheep displayed marked depression, changing appetite, mild to moderate recurrent tympany, dryness of the muzzle and intermittent diarrhea. Muscle tremors, nervous derangement, staggering and frequent recumbency were also evident. Ruminal atony and signs of abdominal discomfort were recorded in all cases.

Values of hematocrit, BUN, SDH, GGT, ALK and AST were significantly elevated associated with reduced Cl level in the diseased sheep; meanwhile the remaining blood biochemical parameters showed non-significant variations from the base values. Changes in acid-base parameters (blood pH, H_2CO_3, TCO_2 and PCO_2) were evident and indicated that, metabolic alkalosis and respiratory compensation had taken place.

Biophysical and microscopical examinations of the rumen liquor were performed, and the effect of the disease condition on the rumen microbial ecosystem was recorded. Total counts of rumen protozoa, microbial activities and numbers of Entodinium species were significantly reduced. On the contrary, Isotricha recorded a significant elevation in the same animals. Other protozoal species showed non-significant changes from the base values. Ruminal fluid sodium and ammonia concentrations increased significantly , while potassium concentration was significantly reduced in the same cases. Medical intervention had taken place, and complete recovery was evident.

INTRODUCTION

Most of the substances entering the rumen are metabolized by ruminal microbes and converted

into nutrient which can be used by the host animal. The ruminal microorganisms may serve as a first line of defense against toxic materials, as these organisms may act to metabolize many naturally occurring toxins in feeds and convert it to substances which do not pretend a threat to the animal health (Reid, 1973). Some other compounds, however, when entering the rumen are metabolized to form substances which are toxic to the host animal. Still other compounds in feeds inhibit or stimulate the activities of rumen microorganisms; causing dramatic changes in the ruminal fermentations (Allison, 1988). These types of metabolic activities may cause digestive disorders and pathological syndromes that have profound negative effects on the health of the host animal.

The rumen microbial ecosystem is maintained in balance by a frequent introduction of the nutrients and by the physiological regulations provided by the animal. However, sudden changes in the diet, or excessive intake of certain types of nutrients, can disrupt the normal microbial balance in the rumen and be detrimental to normal rumen function and to the health of the host animal (Allison, 1988).

One of the unique characteristics of ruminant animals is their ability to acquire tolerance to increased concentrations of toxic materials in feeds. In some cases, this acquired tolerance can be related to changes in population of rumen microbes that lead to increase rates of toxin degradation (Dawson & Allison, 1988). Many of transformations involve reductive reactions or hydrolytic activities of proteases.

Sudden changes in the dietary constituents with consumption of excess amounts of protein-rich concentrates and/or non-protein nitrogenous compounds has resulted in frequent occurrence of rumen alkalosis in ruminants (Randhawa et al., 1989). The disease is characterized by excessive production of ammonia in the rumen which may produce gastrointestinal, hepatic, renal, circulatory and nervous disturbances (Chalupa, 1968; Parkins et al., 1973 and Davidovich et al., 1977).

Even though such studies have been conducted in cattle and much informations are available from the nutritional point of view, little is known regarding the effects of such disease on blood biochemical parameters, liver enzymes, blood gas and rumen microbial ecosystem in sheep. Hence the present study was undertaken to declare and correlate the clinical findings, serum biochemical alterations and biophysical alterations as well as biochemical changes in rumen liquor in induced rumen alkalosis and putrefaction in sheep.

MATERIALS AND METHODS

Animals

Ten apparently healthy ewes, 1.5-2 year old, and weighing between 45-50 kg were used for this study. All animals were carefully observed for an initial period of two weeks before the induction of

the disease. During that period, they were subjected to detailed physical and clinical examination and all findings were recorded for each animal. A balanced maintenance diet consisting of commercial concentrates (cotton seed cake, bran, corn) and hay as well as water was provided ad. libitum.

The Experimental Protocol

Before induction, the ewes were fasted for 12 hrs. and then allowed to overingest low quality, deteriorated feed stuffs including cabbage, turnip and straw pedding previously treated with ammonia and contaminated with fecal matter and urine for a period of 24 hrs until the clinical signs of rumen alkalosis have been observed.

Samples And Sampling Protocol

Samples of rumen liquor and blood were simultaneously collected from each animal in the morning hours (8 AM) before first feeding. The samples were obtained in three occasions, one at zero time from the healthy sheep (base or control), second from the diseased sheep 6 hrs from the onset of the clinical signs, and third from the recovered sheep 72 hrs after treatment.

Venous blood samples were collected by jugular veinpuncture, whereas rumen liquor were drawn by the aid of stomach tube and manual vacum pump. Anticoagulated blood samples were obtained for determination of Hb, PCV, blood gas, blood pH and bicabonate levels. While whole blood samples were obtained for biochemical analysis . (Coles, 1988).

Each rumen sample was divided into two portions. The first portion was sieved and then centrifuged for 15 minutes at 5000 RPM; and only clear supernatant fluid was used for biochemical analysis. The remaining portion of each sample was used to carry out the biophysical and microscopical examinations (Dirksen and Smith, 1987).

Samples Processing

- Biochemical analysis of blood sera and ruminal fluid:

The concentrations of the estimated parameters were measured using the coloremetric analysis system with commercially available test kits supplied by BioMerieux Laboratory/France and Stanbio Laboratory, Inc/USA, according to the methods described by Skeggs and Hochstrasset (1964); Tietz (1970); Belfield and Goldberg (1971); Dummas and Biggs (1972) and Henry et al., (1974).

A microhematocrit with a capillary tube reader was used to determine PCV; hemoglobin was measured in whole blood colorimetrically using a cyanomethemoglobin method (Drabkin & Austin, 1935). For venous blood gas analysis (pH, PCO2), H2CO3 and TCO2, an automated blood gas analyzer (Acid-base Analyzer, Radiometer, Copenhagen, Denmark) was used for these measurements.

• Biophysical and microscopical examinations of the rumen liquor:

pH of rumen liquor was measured by using pH meter (Orion, model SA 720, germany). Color, odor, consistency, sedimentation & flotation test and methylene blue reduction time, all had been examined immediately after collection of samples (Alonso, 1979; Dirksen & Smith, 1987; Roussel, 1990 and House et al., 1992). The total and differential counts of rumen protozoa had been carried out. Protozoal identification was carried out by microscopic examination of stained slides with Lugol's iodine 1% and methyl green , and the protozoa were classified according to the size of the cell, type and location of the cilia, macro- and micronucleus, skeletal plates, and caudal spines and projection of the cuticle. (Hungate, 1966; Church, 1988 and Williams & Colemans, 1988).

• Treatment regimen

The diseased sheep were subjected to oral administration of 150-200 ml of 2.5% kitchen vinegar, Oxytetracycline (Amoxy-Vet tablet R) at a dose rate of 20 mg/kg body weight for three successive days. In addition, 50 gm molasses, 100 ml liquid paraffin, and a mixture of minerals and amino acids (Tonovet R) were used and given orally through stomach tube. This treatment regimen had been administered once/day for three successive days until the animals were completely recovered.

• Statistical analysis

The mean values and standard errors were calculated for all variables. The differences between the mean values were assessed for significance by using Paired Student's t test using the Statview V. 4.01 statistical package for Macintosh computer (Feldman et al., 1994).

RESULTS and DISCUSSION

When the ruminant animals are switched from forage to a high -grain diet or from high quality to low grade fibers, the ruminal microbial population undergoes a dramatic shift.

Before induction of the disease, all animals were active, bright, exhibited normal appetite and had normal respiratory (20-30/m) and heart rates (70-90/m) and normal rumen activity (3-4/2m). Sheep afflicted with this type of reticulorumen disturbances displayed changing appetite, marked depression, dullness, dryness of muzzle, mild to moderate relapsing tympany and intermittent diarrhea. Some animals had muscle tremors particularly in hind quarters and showed signs of nervous derangement. These findings were accompanied by conditions resembling paresis associated with staggering gait and frequent recumbency. Complete loss of rumination, ruminal atony and signs of abdominal discomfort were also observed.

Ruminal atony that observed in this study could be attributed to the significant increase in the ruminal pH and ammonia level, in addition to the

accumulation of toxic amines in the rumen liquor (Verma and Ganapathy, 1973; Randhawa et al., 1989).

Recurrent tympany, abdominal discomfort and intermittent diarrhea observed in the present investigation could be ascribed to the caustic nature of the rumen liquor, stagnated food material that may cause congestion, inflammation and exfoliation of the rumen and digestive tract (Sethuraman and Rathor, 1979). It was also reported by Hoflund (1967) that, the abnormal products of protein breakdown formed by multiplication of coli and proteus bacteria cause gastroenteritis, diarrhea, loss of condition, relapsing tympany and frothing of the rumen contents.

Moreover, Radostits (1994) endorsed these findings and stated that, stagnation of ingesta in the reticulorumen caused putrefaction of the content and appearance of the enteric group of pathologic microbes causing abomasitis and gastrointestinal disturbances.

The nervous derangement with the resultant neurological disorders observed in the diseased ewes are considered to be due to the effect of increased ammonia concentration in decreasing the magnititude and potency of inhibitory post-synaptic potential in the central nervous system. It also might be attributed to the depletion of brain energy due to the subsequent cellular biochemical changes as a result of ammonia shunting across the liver and systemic circulation (Randhawa et al., 1989). This explanation is endorsed by Visek (1966) and Bartley (1976) who reported that, ammonia exists as NH_3 at high pH and as ammonium ion NH4+ at lower pH. Because tissue membranes are permeable to the lipid-soluble NH3 form and impermeable to the charged NH4+ form, absorption of ammonia will be greater at a high rumen pH than at a low pH.

Clinical depression and dullness observed in the diseased ewes might have been caused, at least in part, by decreased food & water intake and increased accumulation of toxic amines in the gastrointestinal tract (Radostits et al., 1994; Ward et al., 1994).

The results of hemogram and biochemical analysis of blood sera are presented in table (1), the mean values of blood pH, blood gas and acid-base are listed in table (2). Meanwhile, the results of biophysical and biochemical analysis of rumen liquor as well as the total & differential count of rumen protozoa are summarized in tables (3&4).

The mean values of packed cell volume and Hb concentrations increased from 34.6±2.21 and 9.76±1.21 up to 41.2±1.16 and 10.05±1.06, respectively at 6 hours from the onset of the clinical signs. These observations are coincided with that obtained by Davidovich et al., (1977) and Randhawa et al., (1989). These elevations could be ascribed to the presenting stress associated with disturbances in the reticulorumen compartment and subsequent alterations in the blood biochemical parameters that result in release of stored erythrocytes into the peripheral

Table (1): Mean values (± SE) for Hb, PCV and serum biochemical parameters in healthy and diseased sheep before and after treatment

	Hb gm/dl	PCV %	Na+ mmol	K+ mmol	Cl- mmol	BUN mg/dl	Tp g/dl	Alb g/dl	Glob g/dl	SDH IU/l	GGT IU/l	AST IU/l	Alk IU/l	Creat. mg/dl
Healthy sheep (control)	9.76a ±1.21	34.6 a ±2.21	168.0 a ±1.41	5.01 a ±0.41	91.5 a ±2.6	15.25 a ±0.95	5.14 a ±0.63	3.0 a ±0.20	2.14 a ±0.13	15.54 a ±1.01	49.7 a ±6.03	±192.6 a ±12.91	217.8 ab ±14.21	0.5 a ±0.0
Diseased sheep before treatment	10.05 a ±1.06	41.2 b ±1.16	166.8 a ±5.87	4.84 a ±0.28	80.5 b ±3.12	18.52 b ±0.78	5.84 a ±0.63	3.02 a ±0.36	2.82 a ±0.46	28.76 b ±2.67	61.4 b ±8.21	237.4 a ±7.02	250.4 b ±12.89	1.0 b ±0.0
Diseased sheep after treatment	9.66 a ±1.77	34.8 a ±1.41	167.6 a ±3.55	4.94 a ±0.15	84.6 b ±1.13	17.24 ab ±1.36	5.28 a ±0.36	3.0 a ±0.26	2.28 a ±0.12	19.96 a ±2.27	53.0 ab ±2.62	233.6 ab ±12.23	200.7 a ±13.76	0.9 a ±0.0

Means with different superscript in the same column are significantly different, while means with the samesuperscript are not significantly different at 5% level of probability

Table (2) : Mean values (± SE) for blood pH, blood gas and bicarbonate in healthy and diseased sheep after treatment

	Blood pH	PCO_2 MMHG	H_2CO_3 mmol/l	TCO_2 mmol/l
Healthy sheep (control)	7.38 a ±0.10	45.86 a ±1.14	26.34 a ±1.16	20.23 a ±1.99
Diseased sheep before treatment	7.50 a ±0.07	50.04 b ±1.11	32.32 b ±1.41	25.6 a ±2.20
Diseased sheep after treatment	7.36 a ±0.07	45.82 ab ±2.16	27.36 ab ±2.74	23.83 a ±1.93

Means with different superscript in the same column are significantly different, while means with the samesuperscript are not significantly different at 5% level of probability

circulation (Davidovich et al., 1977). It also might be referred to the degree of dehydration which may result from reduced food & water intake and diarrhea. Total plasma protein showed non-significant elevation in the diseased sheep, the result which could be explained on the basis of dehydration (Ward et al., 1994).

The pre-renal azotemia (indicated by increased BUN and creatinine) observed in the present investigation might have resulted from hemoconcentration, reduced glomerular filtration and subsequent accumulation of these toxic by-products (Smith et al., 1992; Fouda, 1995). The increased activity of the kidneys in cases of metabolic alkalosis caused severe degeneration followed by necrosis of the tubular epithelium with eventual reduced glomerular filtration (Sethuraman and Rathor, 1979).

It was also mentioned by Carlson (1997) that starvation or other processes that result in rapid tissue catabolism may result in moderate increase in BUN concentration. Moreover, an important point is that many disorders such as colic, peritonitis, acute enteritis and massive blood loss, all initiate the release of vasoactive mediators, which may produce renal damage and impaired renal function.

The obtained results revealed hypokalemia, although the reduction in K+ concentration was not significant; such reduction may be attributed to anorexia, urinary loss and intracellular movement of potassium due to developing alkalosis (Svendsen, 1969; Adrogue & Madias, 1981). This finding is supported by Carlson (1997) who stated that hypokalemia is most commonly seen with altered intake and absorption and with excessive losses from gastrointestinal tract such as in diarrhea.

It is very well known that alterations in chloride concentration are usually associated with proportional changes in sodium concentration as a result of changes in relative water intake. Additionally, chloride concentration tends to vary inversely with bicarbonate concentration (Carlson, 1997). The principal cause of hypochloremia observed in our cases was due concluded to be increased urinary loss and decreased intake during the period of the experiment (Ward et al., 1994). There were no essential changes in the concentration of sodium, the result that agreed with that reported by Papadopoulos et al., (1985).

The obtained results of blood gas and acid-base revealed a significant increases in blood pH, HCO_3 and PCO_2 in the diseased ewes. Such variations in PCO_2 and total CO_2 indicated that there was a respiratory depression and concurrent respiratory compensation (Papadopoulos et al., 1985; Avery et al., 1986). It is worthy mention that, the increase in PCO_2 values was apparently due to increased blood pH, which depresses respiration and ventilation of the alveolar spaces, resulting in rise of PCO_2 of alveolar air and blood. The changes in total CO_2 is highly correlated to changes in PCO_2 in the diseased ewes (Papadopoulos et al., 1985).

Table (3): Biophysical characteristics and Mean values (± SE) for biochemical analysis of ruminal fluid in healthy and diseased sheep before and after treatment

	Color	Odor	Consistency	SAT minute	pH	MB/RT minute	Protozoal activity	Na+ mmol/1	K+ mmol/1	Cl- mmol/1	NH_3 mmol/1
Healthy sheep (control)	olive green	aromatic	slimy	24.8 ± 1.53	6.69 ± a 0.23	7.3 ± a 1.10	+++	125.0 ± a 2.09	29.2 ± a 1.17	17.0 ± a 1.89	93.3 ± a 5.81
Diseased sheep before treatment	dark green to black	offensive or foul	aqueous	foamy	8.11±b 0.34	15.8±b 1.83	+	141.2±b 6.11	23.7 ±b 1.09	17.6 ± a 1.49	142.4 ±b 9.40
Diseased sheep after treatment	olive green	aromatic	slimy	22.7 ± 1.48	6.77± ac 0.24	7.4± ac 0.92	++	128.0± ab 3.66	26.76±ab 1.30	16.4 ± a 2.05	110.25±ac 4.16

Means with similar superscript in the same column are not significantly different, while means with different superscript are significantly different at 5% level of probability.

SAT = Sdimentation and floatation test. MB/RT = Methylene blue reduction time. (++) = Motile and moderate protozoal numbers. (+++) = Highly motile and abundant protozoa. (+) = Sluggish and few protozoal number

Table (4): Mean values (± SE) for total and differential counts of rumen protozoa in healthy and diseased sheep before and after treatment

	Total count x103/ml	Holotrichs %			Entodiniomorphidae (Oligotrichs) %		
		Isotricha	Dasytricha	Entodinium	Epidinium	polyplstron &Ostracodinium	Ophryoscolex
Healthy sheep (control)	903.0 ± a 38.74	13.3 ± a 1.24	0.57 ± a 0.10	73.2 ± a 2.21	12.2 ± a 1.46	0.42 ± a 0.12	0.37 ± a 0.11
Diseased sheep before treatment	608.0 ± b 62.73	19.1 ± b 1.75	1.04 ± a 0.21	65.4 ± b 1.82	13.7 ± a 2.28	0.61 ± a 0.16	0.46 ± a 0.14
Diseased sheep after treatment	738.0 ± b 50.13	15.0 ± ab 2.0	0.66 ± a 0.10	70.9 ± ab 4.52	12.3 ± a 1.49	0.39 ± a 0.11	0.37 ± a 0.09

Means with the same superscript in the same column are not significantly different, while means with different superscript are significantly different at 5% level of prbability.

Biochemical analysis of blood sera revealed significant elevations in the levels of liver enzymes in the post-induction period. These elevations are ascribed to the escape of these enzymes from the disrupted hepatic cells with necrosis or altered permeability of the cell membranes (Sethuraman and Rathor, 1979; Randhawa et al., 1989). The pre- and post-induction levels of aminotransferase, alkaline phosphatase, sorbitol dehydrogenase and gama glutamyle transferase are in agreement with the results of Bartley et al., (1976), Randhawa et al., (1989), Caballero et al., (1992) and Fouda et al., (1995).

The concentrations of sorbitol dehydrogenase increased significantly from the preinduction level of 15.54±1.01 to 28.76±2.67 U/L in the diseased ewes. This elevation could be referred to the damaged liver cells associated with absorption of bacteria and/or toxins from the damaged bowel into the portal circulation (Carlson, 1997).

The recorded ruminal pH increased significantly from 6.69±0.23 in healthy sheep to 8.11±0.34 in the diseased ones. These results are in agreement with that reported by Hoflund (1967). Such increase in ruminal pH could be referred to poor buffering capacity of the rumen fluid against the increased alkali (Randhawa et al., 1989), or to the intensified production of more or less toxic alkaline products of the breakdown of protein with eventual increase in the rumen pH up to 8.5 (Hoflund, 1967).

In the present investigation, the rumen fluid had a black-green color, foul smell with an aqueous to foamy consistency; the results that agreed with those of Hoflund (1967), Alonso (1979) and Dirksen (1983). These findings have been resulted from stagnation of the ingesta in the reticulorumen, and putrefaction caused by the enteric group of microbes such as coli and proteus (Radostits et al., 1994).

The redox potential as measured by methylene blue reduction time is inversely proportional to the microbial activity. Relatively, indigestible diet such as straw, inanition and reticulorumen disorders, all result in considerable delay in the reaction (Dirksen and Smith, 1987), the condition that observed in the present study which revealed significant reduction in the total protozoal population and their activity. These results are in the same concern with that reported by Hoflund (1967) who stated that, in cases of putrefaction of the reticulorumen contents, the infusoria died out, the saprophytic bacteria increased and the production of volatile fatty acids reduced. Such reduction in the total numbers and activities is ascribed to the caustic effect of increased rumen pH, accumulation of toxic amines and consequently disturbed rumen ecosystem (Dirksen, 1983).

The differential rumen protozoal counts revealed a significant increase in the numbers of Isotricha associated with significant reduction in the numbers of Entodinium species in the post-induction period; other protozoal species showed non significant variations between the

diseased and healthy sheep. The results are endorsed by those of Alonso (1979) who concluded that Isotricha is the most common family of Holotrichs, which peculiarly become more numerous and active under the adverse conditions. The results also agreed with those obtained by Clarke & Reid (1972) and Ffoulkes & Leng (1988) who supported the point that, the rumen protozoa are mainly large holotrichs in animals fed on green pastures.

The rumen fluid sodium concentration showed significant increase in post-induction period, while the concentration of potassium reduced significantly in the same period associated with non-significan t variations in the level of rumen fluid chloride.

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