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EFFECT OF ACUTE RUMINAL ACIDOSIS ON RIBOFLAVIN AND NIACIN CONCENTRATIONS IN SHEEP

Abdullah Başoğlu [1] Kürşat Turgut [1] Mursayettin Eksen [2] Bünyamin Traş [3]
Mehmet Maden [1] Mahmut Ok [1] A. Levent Baş [3] Tufan Keçeci [2]

Koyunlarda Akut Ruminal Asidozisin Riboflavin ve Niacin Konsentrasyonlarına Etkisi

Summary: *Six non-lactating, non-pregnant adult ewes divided into two groups of 3 animals each as a control and experimental groups were used to evaluate the effect of experimentally induced acute ruminal acidosis on plasma and rumen fluid riboflavin and niacin concentrations. Decreasements in ruminal fluid pH, the count of protozoa and increase in the count of bacteria were related to gradual decreases in plasma riboflavin and niacin concentration. Plasma riboflavin and niacin concentrations decreased respectively from 9.51 mcg/ml and 7.01 mcg/ml to 0.42 mcg/ml and 0.09 mcg/ml at the first day of the experiment (p<0.05) and than inclined towards normal values. Similar drastical decrease in the niacin concentration of rumen fluid was determined at the first day of the experiment.*

Özet: *Bu çalışma deneysel olarak oluşturulan akut ruminal asidozisin plazma ve rumen sıvısı riboflavin ve niasin konsantrasyonlarına etkisini araştırmak amacıyla, laktasyonda ve gebe olmayan altı koyunda yapıldı. Rumen sıvısı pH'sı ve protozoa sayısındaki azalış ile bakteri sayısındaki artış, plazma riboflavin ve niacin konsentrasyonlarındaki dereceli azalış ile ilgiliydi. Denemenin birinci gününde, plazma riboflavin ve niacin konsantrasyonları sırasıyla 9.51 ve 7.01 mcg/ml'den 0.42 ve 0.09 mcg/ml'ye düştü (p<0.05) ve sonraki günlerde normal değerlere doğru yükseldi. Benzer şekildeki ani ve şiddetli bir düşme rumen sıvısı niasin konsantrasyonlarında da gözlendi.*

Introduction

It is well known that the microorganisms in the rumen synthesize B vitamins (9). For years, it was commonly accepted that ruminants did not need supplementation of B-complex vitamins because of the fact that the rumen microflora synthesized the vitamins in sufficient quantities to fulfill the host's requirements (1, 19). However it is now known that under certain conditions, cattle and sheep do respond to extra diatery B vitamins. Factors affecting B vitamins requirements are many. Diseases affecting metabolic and digestive efficiencies are the most common factors causing B vitamins requirements (3, 16).

Ruminal acidosis is a metabolic disease of ruminants which is charactarized by ruminal and generalized systemic acidosis resulting in anorexia, weakness, prostration, coma and frequently death. In ruminal acidosis, there is an initial decrease in rumen pH primarily due to an increase in organic acids. As the pH is lowered, there is a change in the rumen microflora. First, the number of protozoa begin to decrease, then there is a decrease in the number of normal gram negative bacteria. These bacteria are replaced by an increased number of gram positive organisms (St. bovis). The changes cause the impairment of the B vitamins synthesis (8).

Phillipson and Raid (18) stated that Streptococci which are rapidly overgrown in ruminal acidosis consume thiamine which may be a factor in the development of subsequent neurologic signs. Additionally, alteration in the normal rumen microflora where microorganisms such as Cl. sporogenes or B. thiaminolyticus produce thiaminase in ruminal acidosis (2, 22). For that reason, the use of Thiamine hydrochloride is considered as a part of the treatment of ruminal acidosis to help prevent poliencephalomalacia (8). Howard (8) also stated that B-complex vi-

1. S.Ü. Veteriner Fakültesi, İç Hastalıkları ABD.
2. S.Ü. Veteriner Fakültesi, Fizyoloji ABD.
3. S.Ü. Veteriner Fakültesi, Farmakoloji ABD.

tamins might be given orally or parenterally on an amprical basis in ruminal acidosis. So, the necessity of B vitamins suplementation apart from Thiamine has recieved little attention.

The present works was undertaken to study the effect of experimentally induced acute ruminal acidosis on the riboflavin and niacin concentrations of plasma and rumen fluid to provide unique insight on the suplementation of these vitamins in the case of acute ruminal acidosis.

Materials And Methods

Experimental animals:

Six, non lactating, non pregnant adult ewes, body weight mean 48 kg (range 41-58) were used. The animals were divided into two groups of 3 animals each as a control group and experimental group on which ruminal acidosis was performed. Two weeks before and during the experiment, each group of ewes were kept in individual metabolism cages. The animals were fed mixed grass-alfa alfa hay and concentrated ration according to N.R.C. (15) requirements twice in a day at 9.30 a.m. and 17.30 p.m. Fresh top water was continually on offer.

Experimental procedure:

To establish normal values, samples of rumen fluid for the determination of niacin concentration, ruminal fluid pH and protozoa and bacteria counting, and venous blood samples (heparinized) for the determination of plasma riboflavin and niacin concentrations were collected from each animal before feeding at 8.00 a.m. on two occasions before the experiment. The same sample collections were performed during the experiment. The same sample collections were performed during the experiment in both control and experimental groups of ewes every day at 8.00 a.m. for five consequtive days.

To produce acute ruminal acidosis, on day 0 at 24 p.m. all ewes in experimental group were given 50 gram of wheat flour perkilogram of body weight. The flour was given as a suspension in lukewarm water (1:2) by stomach tube.

Clinical examinations and treatments:

At each sampling time, all animals were examined with regard to appetite, rumination, diarrhoea, general apperance and behaviour. Treatment of the ewes in the experimental group was started when the symptoms of the central nervous system depression became severe (blood pH 7.00±0.20 and base deficit-15±3). In the treatment, no oral treatment was applied, only intravenous fluid therapy with isotonic sodium bicarbonate and saline solution described by Howard (8) was given to the experimental group of ewes every day after sampling. In addition to this, Antihistamine 300 mg subcutaneously was administered three times per day for 3 consequtive days as a supportive treatment.

Analytical techniques:

Rumen fluid pH determination was measured by means of electronic pH meter (ACT pH meter, Piccola Model. Singapore). The counts of the protozoa and bacteria of ruminal fluid samples were determined by the method described Eksen et al (5). Riboflavin and niacin concentrations of the plasma samples and niacin concentration of the rumen fluid samples were analysed by HPLC (Shimadzu-LA-6A).

Extraction procedure was carried out according to Ichinose and Adachi (11) and measurement were performed according to Vandemark (23).

Statistical analysis:

Student t test for independent means was used to assess any differences between control and experimental groups (20).

Results

Clinicanl findings:

On day 1, all animals showed CNS depression, developed watery diarrhoe, and exhibited periods of trembling. All were able to stand up, however mostly with their head and tail kept in a low position. Rumination was not observed to take place in any of the animals during day 1-3. Parallel to this, there was no appetite recorded during this period on day 3 all animals showed little interest in food and water. Laminitis was observed in 2 sheep at the 3th day of the experiment.

Laboratory findings:

The mean pH values and the counts of protozoa and bacteria of the ruminal fluid samples in both control and experimental groups of ewes and their variations during the experiment are presented in figure 1.

The mean pH values of the rumen fluid samples of the experimental group dropped from 6.84 to 4.76 at the first day of the experiment ($p<0,05$), and then inclined towards the normal values. The counts of the protozoa also decreased gradually from $240x10^3$/ml to $4x10^3$/ml by the 5th day of the experiment. The diffrences were significant ($p<0,05$) after the first day of the experiment. However, the counts of the bacteria incrased drastically from $11,28x10^9$/ml to $17,41x10^9$/ml at the first day of the experiment ($p<0,05$) and then fluctuated, but always stayed high.

The mean plasma riboflavin and niacin concentrations and rumen fluid niacin concentrations in both control and experiment al groups of ewes and their variations during the experiment are represented in figure 2. Plasma riboflavin and niacin concentrations decreased respectively from 9,51 mcg/ml and 7,01 mcg/ml to 0,42 mcg/ml and 0,09 mcg/ml at the first day of the experiment ($p<0,05$) and then inclined towards normal values. Similar drastical decrease in the niacin concentration of rumen fluid was determined at the first day of the experiment ($p<0,05$).

Figure 3 shows chromatograms of riboflavin and niacin in standart solutions and plasma samples.

Discussion

In this study, a drastical decreases in the riboflavin and niacin concentrations of plasma and in the niacin concentration of ruminal fluid was observed after experimental induction of acute rumen acidosis and then the concentrations of these vitamins inclined towards normal values.

It has been reported that the counts of the protozoa in the rumen fluid is directly related with the pH values of the rumen fluid; while the pH decreases, the counts of the protozoa also decreases (12, 20). The mean rumen fluid pH value of the experimental group of ewes dropped from 6,84 to 4,76 at the first day of the experiment and then, inclined towards normal values. The counts of the protozoa also decreased gradually from 240 x 10^3/ml to 4 x 10^3/ml by the 5th day of the experiment.

There is a negative correlation between the counts of the bacteria and the counts of the protozoa in the rumen (4, 24) which is in accordance with the result of the present study. The counts of the protozoa in ruminal fluid decreased gradually from a normal $240x10^3$/ml to $4x10^3$/ml by the 5th day of the experiment, whereas, the counts of the bacteria increased drastically from a normal $11,28x10^9$/ml to $17,41x10^9$/ml at the first day of the experiment and then fluctuated, but always stayed high.

There is net synthesis of B vitamins in the rumen (17, 19) and it is commonly accepted that the rumen bacteria play a great role in this synthesis (5, 14, 17). However, some research workers have stated that the protozoa in the rumen also took part into this synthesis (6, 10). According to the result of this study, it is rather difficult to clarify which group of microorganism of the rumen plays a role in the synthesis of B vitamins. Decreaments of riboflavin and niacin concentations could be either the lack of the sufficient number of protozoa or the change of the bacterial flora of the rumen. In this study, plasma riboflavin and niacin concentrations decreased respectively from 9,51 mcg/ml and 7.01 mcg/ml to 0,42 mcg/ml and 0,09 mcg/ml at the first day of the experiment and then inclined towards normal values. Similar a gradual and significant desrease in the plasma thiamine concentration has been reported in a study experimentally induced rumen acidosis (21). Decreaments in the riboflavin and niacin concentrations of plasma could be ascribed to inadequate synthesis of these vitamins in the rumen due to changed microbial pattern in the acidotic rumen.

The low niacin content of plasma in acidotic ewes was directly related to its low level in the rumen fluid. Similar relationship between plasma and ruminal fluid thiamin concentrations has been reported in acidotic buffalo calves (21). Concentrations of B vitamins in the ruminal fluid represent a balance between additions through feed intake and microbial synthesis and removal by absorbtion from the rumen, passage to the abomasum, utilization by microorganisms and possibly chemical or microbial inactivation (7). So, changes in the type of the microorganisms in the rumen, impaired absorbtion or increased metabolic demand for riboflavin and niacin in the absence of inceased supply associated with increased urinary excretion of these vitamins may cause the decrease in riboflavin and niacin levels. Increaments in the ruminal fluid niacin and plasma riboflavin and niacin concentrations at the second day of the experiment onward in subacute lactic acidosis was due to improvement in the dietary intake and restoration of microflora of the rumen. This in turn resulted in an increased microbial synthesis of riboflavin and niacin in the rumen due to the shift of ruminal microbial population towards normal level.

In conclusion, the result of the present study showed that riboflavin and niacin supplementation is to be consireder as a part of the treatment of ruminal acidosis and supplementation of these vitamins will help prevent the subsequents deficiencies.

References

1-Agrawala, I.P., Huffman. C.P., Luecke. R.W., Duncan, C.V. (1953). A quantitative study of rumen synthesis in bovine on naturel and purified rations. III. Riboflavine, panthonetic acid and niacin. J. Nutr. 49, 631-658.

2-Bartley, E.E., Brent, B.E. (1984). B vitamins for ruminants. Mn. Nut. Conf. Sep. 20-21.

3-Brent, B.E. (1978). B-vitamins requirements of ruminants. Mn. Nut. Conf.

4-Devuvst, A., Joramillo, D., Vankelle, M., Arnould, R. (1973). Comparison of the effects of different rations on the development of ciliates in the rumen. Moreels, A. Zeitschrift fur Tierphysiologie. Tierernahrung und Futtermittelkunde. 31, 136-149.

5-Eksen, M., Durgun, S., Akmaz, A., İnal, Ş., Şeker, E. 1991). Koyunlarda mikrofaunanın kan metabolitleri, canlı ağırlık artışı, yemden yararlanma ve karkas özelliklerine etkisi. Doğa Tr. J. of Veterinary and Animal Sciences. 15, 207-228.

6-Fairbanks, W.B., Krider, J.L. (1944). Factors involved in the synthesis of vitamins in the ruminant. North. Anim. Veterinarian. 25, 97-100.

7-Have, B.W., Mitchell, G.E., Little, C.O., Bradley, N.W. (1966). Concentrations of B-vitamins in ruminal fluid of steers feed different levels and physical forms of hay and grain. J. Anim. Sci. 25, 539-542.

8-Howard, J.L. (1981). Ruminal metabolic acidosis. The Bovine Practitioner, 16, 44-53.

9-Hungate, R.E. (1966). The rumen and its microbes. New York: Academic Press.

10-Hunt, C.H., Kick, C.H., Burroughs, E.W., Bethke, R.M., Schalk, A.F., Gerlaugh, P. (1943). Studies of riboflavin and thiamine in the rumen contents of cattle, J. Nutr. 25:207-216.

11-Ichinose, N., Adachi, K. (1985). Determination of B vitamins in serum of fish using High-Performance Liquid Chromatography with fluorescence detection. Amalyst. December. 10:1505-1508.

12-Kocabatmaz, M. (1980). Değişik oranlarda şeker pancarı posası kapsayan rasyonların Akkaraman koyunlarda rumen mikrofaunası üzerine etkileri ile rumen içeriği ve bazı kan metabolitleri üzerindeki fizyolojik değişiklikler. TÜBİTAK. VHAG-475.

13-Mead, R., Curnow, R. N. (1987). Statistical methods in agriculture and experimental biology. J. W. Arrow Smith Ltd. Bristol.

14-Morrison, F.B. (1961) Feeds and feeding, abridged. 9th ed. Morrison Publishing. Clinton. IA.

15-National Research Council (1975) Nutrient requirements of sheep. Sth. Rev. Ed. National Academy of Science. Washington D.C.

16-Olentine, C. (1984). B vitamins for ruminants. Issue of feed management. April.

17-Pearson, P.B., Struglia, L., Lindahl, I.L. (1953). The fecal and urinary excretion of contain B vitamins by sheep fed hay and semisynthetic rations. J. Anim. Sci. 12, 213-218.

18-Phillipson, A.I., Reid, R.S. (1957) Thiamine in the contents of the alimentary tract of sheep. Br. J. Nutr., 11, 27-29.

19-Porter, J.W.G. (1961). Vitamin synthesis in the rumen: In Digestive Physiology and Nutrition of the Ruminant. pp. 226-234. Ed. D. Lewis. London. Butterwaths.

20-Purser, D.B., Mair, R.J. (1959). Ruminal flora studies in the sheep IX. The effect of pH on the ciliate population of the rumen in vivo. Austral. J. Agric. Res. 10, 55-564.

21-Randhawa, S.S., Ahuja, A.K., Rathor, S.S. (1988). Effect of lactic acidosis on histamine and thiamine levels in buffalo calves. Indian Journal of Animal Sciences. 58,1019-1023.

22-Streeve, J.E., Eolwin, E.E. (1974). Thiaminase producing strains of C. sporogenes associated with outbreaks of cerebrocortical necrosis. Vet. Rec. 94, 330-333.

23-Vandemark, F.L. (1980) Automated multivitamin analysis by liquid Chromatography. Pittsburg Conference on Analpytic Chemistry and Applied Spectroscopy. March 1980, paper no:309.

24-Warner, A.C.I. (1962). Some factors influencing the rumen microbial population. J. Gen. Microbiol. 28:129-146.

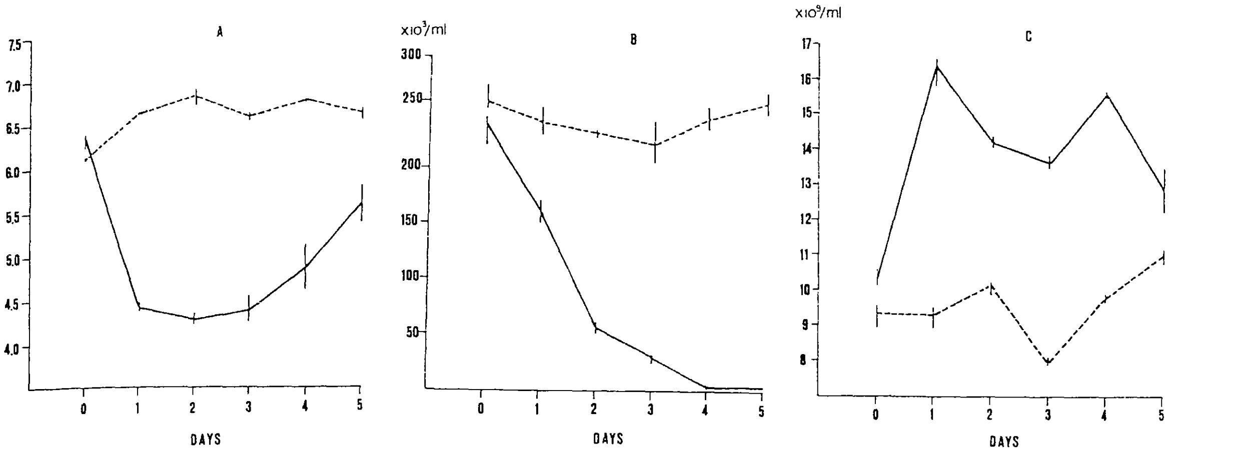

Figure 1. The mean pH values (A) and the counts of protozoa (B) and bacteria (C) of ruminal fluid smaples in both control (----) and experimental (——) groups of ewes and their variations during the experiment.

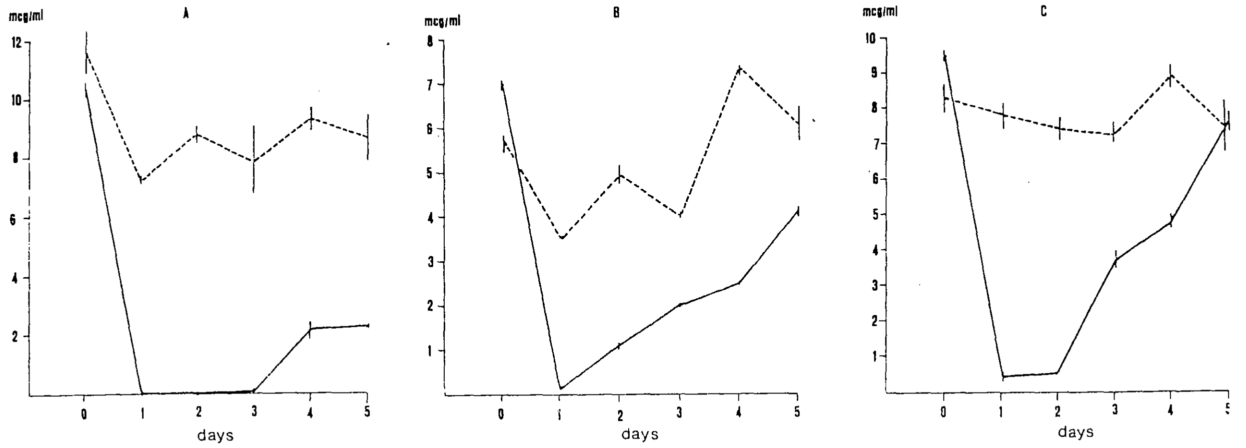

Figure 2. The rumen fluid niacin (A) and plasma niacin (B) and riboflavin (C) concentrations in both control (---) and experimental (—) groups of ewes and their variations during the experiment.

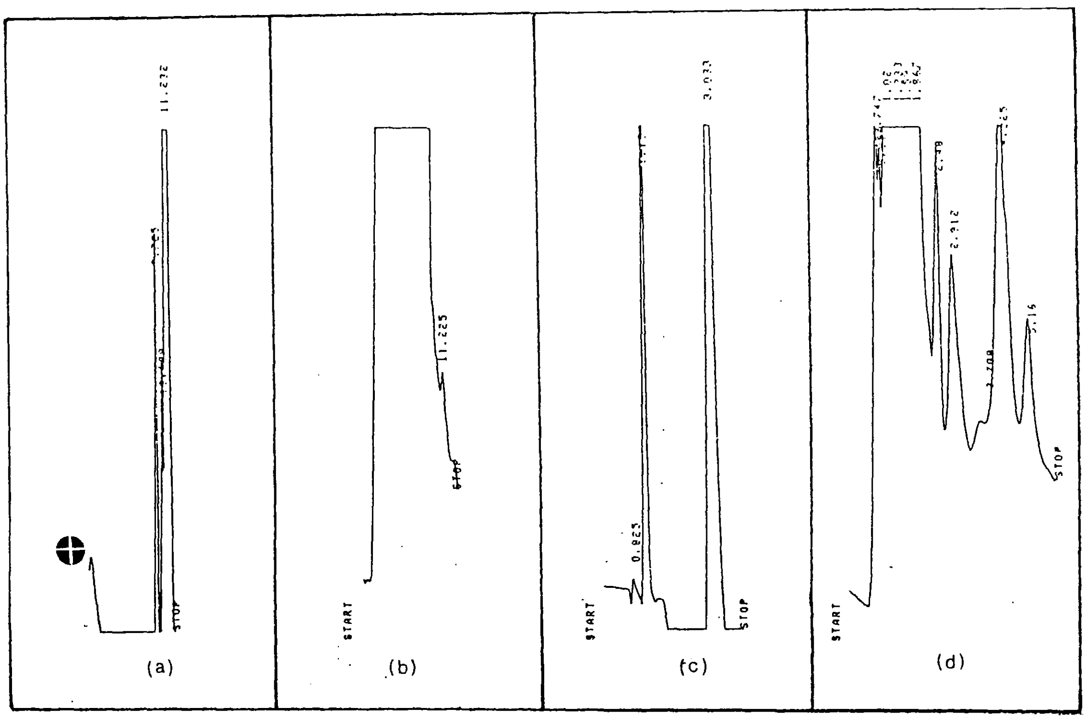

Figure 3. Chromotograms of Riboflavin and Niacin
a) THe standart solution of riboflavin (500 ng)
b) The plasma samples of riboflavin
c) The standart solution of niacin (50 ng)
d) THe plasma samples of niacin