EFFECT OF PHLORHIZIN - INDUCED KETOSIS ON RIBOFLAVIN AND NIACIN LEVELS IN SHEEP

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Koyunlarda Phlorhizinle Oluşturulan Ketozisin Riboflovin ve Niacin Seviyelerine Etkisi

Sunnary : *Six non-lactating, non-pregnant adult ewes divided into two groups of 3 animal each as a control and experimental groups were used to evaluate the effect of phlorhizin-induced ketosis on plasma and rumen fluid riboflavin and niacin concentrations. The mean pH values and the counts of protozoa and bacteria of the rumen fluid in experimental group did not change significantly (p>0.05). Alteration of plasma riboflavin concentration in experimental group was not significant (p>0.05). Whereas both plasma and rumen fluid niacin concentrations decreased from 5.03 mcg/ml and 9.53 mcg/ml to 2.39 mcg/ml and 2.41 mcg/ml respectively and decraements in both plasma and rumen fluid niacin concentrations were significant (p<0.05).*

Özet : *Bu çalışma phlorhizinle deneysel olarak oluşturulan ketozisin, plazma ve rumen sıvısı riboflavin ve niasin konsantrasyonlarına etkisini araştırmak amacıyla, laktasyonda ve gebe olmayan altı koyunda yapıldı. Deneme grubunda rumen sıvısının ortalama pH değerleri, protozoa ve bakteri sayıları ve plazma riboflavin konsantrasyonlarındaki değişiklik önemli değildi (p>0.05). Bununla birlikte plazma ve rumen sıvısı niasin konsantrasyonları sırasıyla 5.03 ve 9.53 mcg/ml'den 2.39 ve 2.41 mcg/ml'ye düştü ve bu düşüşler önemliydi (p<0.05).*

Introduction

For years, İt was commonly accepted knowledge that ruminants did not need supplementation of B complex vitamins because of the fact that the rumen microflora synthesized the vitamins in sufficient quantitaties to fulfill the host requirement (1, 23, 24). However it is now known that under certain conditions, such as metabolic and digestive deficiencies. B vitamins supplementation is a necessity (23, 4, 25).

Ketosis is a metabolic disease characterized by increases of blood, urine and milk ketone bodies concentrations, increases of free fatty acid (FFA) concentration in plasma and decreases of blood glucose concentration.

Numerous research papers have appeared over the last 10 years in which some response to niacine supplementation in cattle and sheep with subclinic and clinic ketosis has been obtained (12, 7, 2).

It has been suggested that deficiencies in B vitamins could make the incidence of ketosis worse (11). There are no reports of B vitamins deficiency showing up as a ketosis problem first. However, cobalt deficiency and therefore, vitamin B deficiency has been implicated as a cause of ketosis (22). Blood and liver levels of vitamin B have been found to be reduced in the postparturient cows (26).

Unfortunately, no chemical data have been reported on the alteration of riboflavin and niacin concentrations in ketosis and the role of these vitamins in the development of ketosis.

Phlorhizin which causes glicosuria have been used in sheep to induce ketonemia and hypoglycemia and has been used as a model in experimental and research works. Subcutaneus injections of phlorhizin increases nonesterified fatty acids and B-hydoxybutyrate and decreases glucose (7, 2).

The objective of this study was to determine alteration of plasma riboflavin and niacin concentrations and rumen fluid niacin concentrations in sheep with phlorhizin induced ketosis and to provide unique insight on the supplementation of these vitamins in the treatment of ketosis.

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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 ketosis was induced by subcutaneus injections of phlorhizin. Two weeks before and during the experiment, each group of ewes were kept in individual metobolism cages. The animals were fed mixed grass-alfa alfa hay and concentrated ration according to NCR (21) 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 and ruminal fluid pH, protozoa and bacteria counting and venous blood samples (heparinized) for the determination of plasma riboflovin and niacin concentrations were collected from each animal before feeding at 8.00 a.m. on two occasion before the experiment in both control and experimental groups of ewes everyday at 8.00 a.m. and at 5, 7, 9, 11, 13 days of the experiment.

In the mean time, concentrations of fasting plasma glucose and Free fatty acids (FFA) were determined for samples obtained on two occasions before the experiment and for the samples obtained at 5, 9, 13 days of the experiment. Urine was also examined for urine glucose and keton bodies every day during the experiment.

To induce ketosis, Phlorhizin was injected to ewes from the beginning to 12 th day. Phlorhizin (Sigma Chemical Co. St. Louis. M.O.) was dissolved in propylene glycol (100 mg/ml) and 300 mg of phlorhizin was given subcutaneously to the ewes twice daily.

Clinical examinations

All animals were examined with regard to appetite, rumination, general appearence subsequently with urine ketone bodies test every day during the experiment.

Analytic 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 were determined by the method described Eksen et al (13).

Extraction procedure for riboflavin and niacin was carried out according to Ichinose and Adachi (14) and measurements were performed according to Vandemark (28).

Fasting plasma glucose concentration was determined by commercial kit (Biobak lab. Supplies trade and Industries inc.). Plasma FFA concentration was determined by a colorimetric method (6).

Urine analyses for glucose and ketone bodies were performed by combined test strips (Combur-6-Test. Boehringer/Mannheim) and Rothera's reagent.

Statistical analysis

Student t test for independent means was used to assess any differences between control and experimental groups (19).

Results

Clinical findings : No abnormal clinical findings was observed in all ewes until the 5th day of the experiment. And then developed progressively were observed. The weight loss for each ewes was about 5 kg at the and of the experiment.

Laboratory findings : Administration of phlorhizin resulted in a additional decrease of fasting plasma glucose with increases in plasma FFA concentration (Figure 1). Fasting plasma glucose concentration decreased from 68.56 mg/dl to 35.17 mg/dl and plasma FFA concentration increased from 0.17 mM/L to 0.91 mM/L in the ewes of experimental group. Both components were significantly different from the values of control group ($p<0.05$).

Urine analysis for ketone bodies were positive at the end of the experiment for the all ewes of experimental group. Administration of phlorhizin caused measurable glucose in urine.

The mean pH values and the counts of protozoa and bacteria of the ruminal fluid in both control and experimental groups of ewes and their variations during the experiment are presented in figure 2. All components were not significantly different from the values of control group ($p>0.05$).

The mean plasma riboflavin and niacin concentrations and rumen fluid niacin concentration in both control and experimental groups of ewes and their variations during the experiment are represented in figure 3. Plasma riboflavin con-

centration was not significantly different from the values of control group ($p>0.05$). Whereas, both plasma and rumen fluid niacin concentrations decreased from 5.03 mcg/ml and 9.35 mcg/ml to 2.39 mcg/ml and 2.41 mcg/ml respectively and decreaments in both plasma and rumen fluid niacin concentrations were significant ($p<0.05$).

Fig. 4 shows chromatograms of riboflovin and niacin in standart solutions and plasma samples.

Discussion

The result of the study showed that both plasma and rumen fluid niacin concentrations decreased significantly ($p<0.05$) in sheep with phlorhizin induced ketosis wherease, plasma riboflovin concentration did not change significantly ($p>0.05$).

Phlorhizin given to sheep subcutaneously decreased blood glucose concentration, increased plasma FFA concentration and caused glucosuria. Phlorhizin decreases blood glucose concentration causing glucose excretion by the kidneys (8, 17). The copious glucosuria results from both competetive and noncompetetive inhibition of renal tubular reabsorbtion of glucose (20). Similar to the result of the present research work it was also found that sheep treated with phlorhizin had decreased blood glucose concentration, increased blood ketone bodies and plasma FFA concentrations (2, 20).

Because the clinical changes of lactation ketosis include hypoglycemia, ketonemia and increased FFA concentration, phlorhizin administrated to sheep provided a model that parallels lactation ketosis.

In this study, plasma riboflavin concentration did not change significantly ($p<0.05$) when compared with the values of control group in spite of decreased feed intake which increased gradually by the time. It has been assumed that riboflavin is synthesized in the rumen contents and it is largely intracellular and little absorbtion occurs from the rumen (9). Riboflavin is the component of the coenzymes flavin mononucleotide and flavin adenine dinucleotide and essential for hydrogen transport (i.e. oxidation phosphorilation) (23). Although riboflavin deficiencies have been demonstrated in young ruminant animals (19). No response to supplemental riboflavin has been reported in animals with a functional rumen.

Plasma and rumen fluid niacin concentrations gradually and significantly decreased in experimental group of ewes ($p<0.05$). Niacin is synthesited in the rumen by the rumen bacteria and is largely intracellular and little absorbtion occurs from the rumen (9, 19). Niacin is a component of hydrogen carrying coenzymes Nicotinamide adenine dinucleotide. Nicotinamide adenine dinucleotide phosphate and essential for hydrogen transport. Biochemical systems using these coenzymes include oxidative deamination : krebs cycle : catabolism of glucose : fatty acid synthesis and oxidation : glycerol synthesis and catabolism : and dehydration of alcohol (23).

Although there are no reports of niacin decrement in the ketosis. Numerous research papers have appeared over the last 10 yerars in which some response to niacine supplementation in cattle with clinical ketosis has been obtained (12, 2, 8).

Supplementation of diets of post partum cows with niacin increased milk production and improved persistency (12) Supplementing cows'diets with niacin 2 weeks before or immediately after calving lowered blood Beta-hydroxybutirate and plasma NEFA concentrations and increased serum glucose concentration (7).

The mechanism by which niacin exerts these changes is not entirely clear. Administration of niacin is known to increase blood glucose concentration in some species (27, 29). In addition, niacin has antilipolytic properties (29). Kronfeld and Raggi (26), postulated that the metabolic disorder in lactation ketosis may arise from a shortage of all forms of nicotinamide adenine dinucleotide coenzymes. Supplementation of postpartum cow diets with niacin beginning 2 weeks before calving prevented the decrease in RBC niacin concentration that was observed in nonsupplemented postpartum cows (7). It is possible that the lack of a decrease in RBC niacin concentration in niacin-supplemented cows could have a systemic physiologic effect by making available greater quantities of nicotinamide adenine dinucleotide coenzymes essential for proper energy metabolism.

The mechanism by which niacin exerts its effect on lipid metabolism is not established. It has been suggested that niacin reduces cyclic AMP (27) either by inhibiting adenyl cyclase activity or by stimulating phosphodiesterase activity (28). The

reduced cyclic AMP would reduce lipase activity sensitive to hormone, resulting in depressed lipolysis by adipose tissue. During ketosis increased hepatic ketogenesis reflects an inability of the liver to oxidize or esterify free fatty acids of a rate comparable to the influx (3). Thus reducing adipose lipolysis would decrease the flux of free fatty acid to the liver, allowing hepatic lipid metabolism to return to normal. Decreased plasma and rumen fluid niacin concentration in experimental group of ewes might be caused by decreased feed intake. Although there are no reports of B vitamins deficiency showing up as a ketosis problem first, according to the result of this study decreased niacin concentration in experimental group of ewes might have contributed to the development of ketosis due to decrease in RBC niacin concentration resulting shortage of all forms of nicotinamide adenine dinucleotide coenzymes and increase cyclic AMP allowing lipase activity sensitive to hormone, resulting in activated lipolysis by adipose tissue.

In conclusion, the result of the present study showed that decreased niacin concentration may contribute to the development of ketosis so niacin supplementation is to be considered as a part of the prevention and treatment of ketosis.

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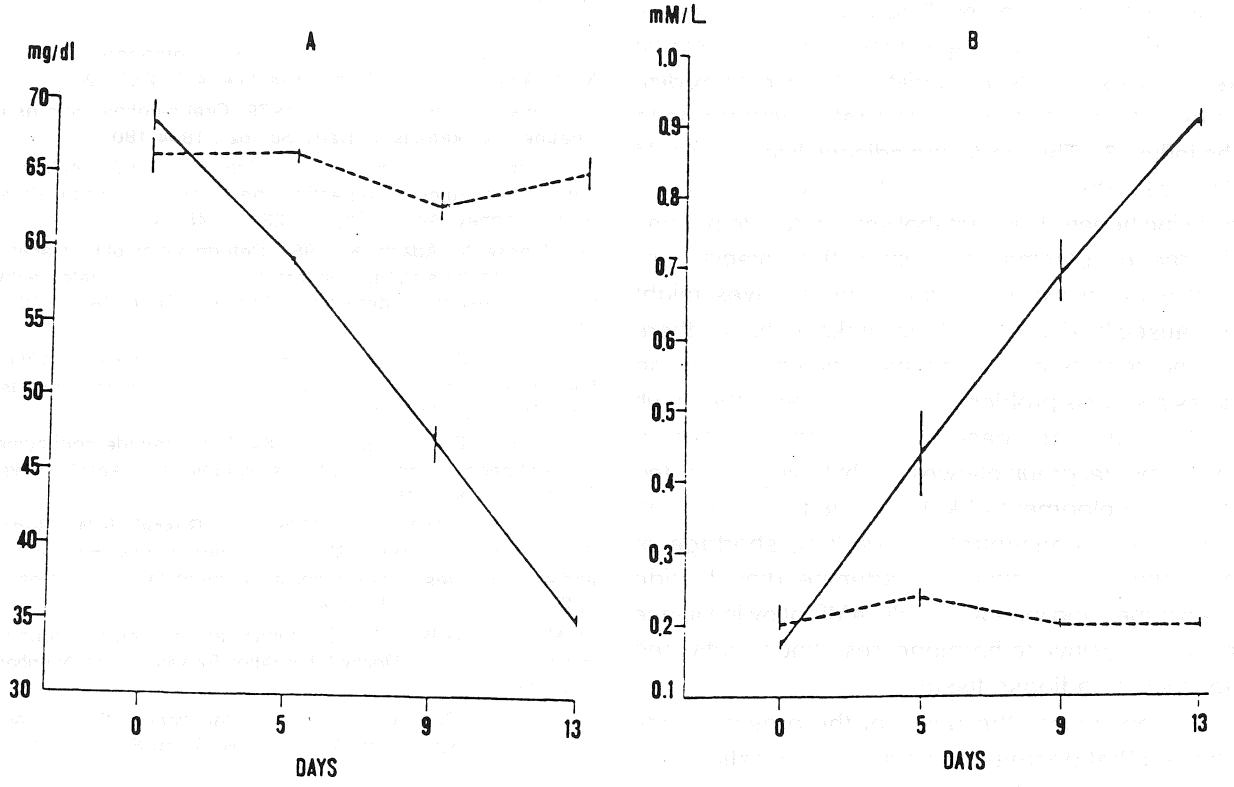

Figure 1. The mean fasting blood glucose (A) and plasma FFA (B) concentrations in both control (---) and experimental (—) groups of ewes and their variations during the experiment.

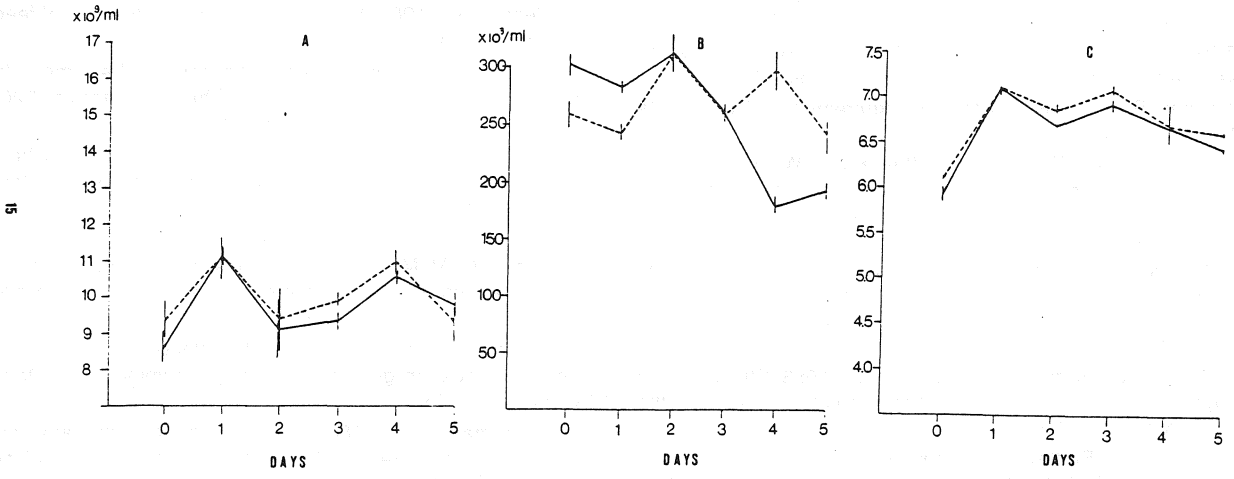

Figure 2. The counts of bacteria (A) and protozoa (B) and the mean pH values (C) of the rumen fluid in both control (---) and experimental (—) groups of ewes and their variations during the experiment.

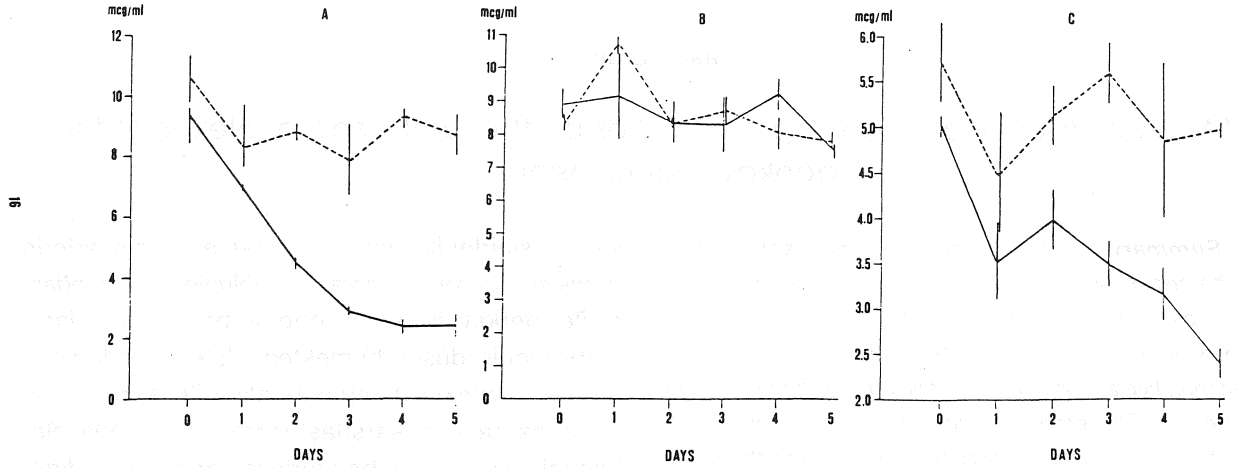

Figure 3. The rumen fluid niacin (A) and plasma riboflavin (B) and niacin (C) concentrations in both control (---) and experimental (—) groups of ewes and their variations during the experiment.

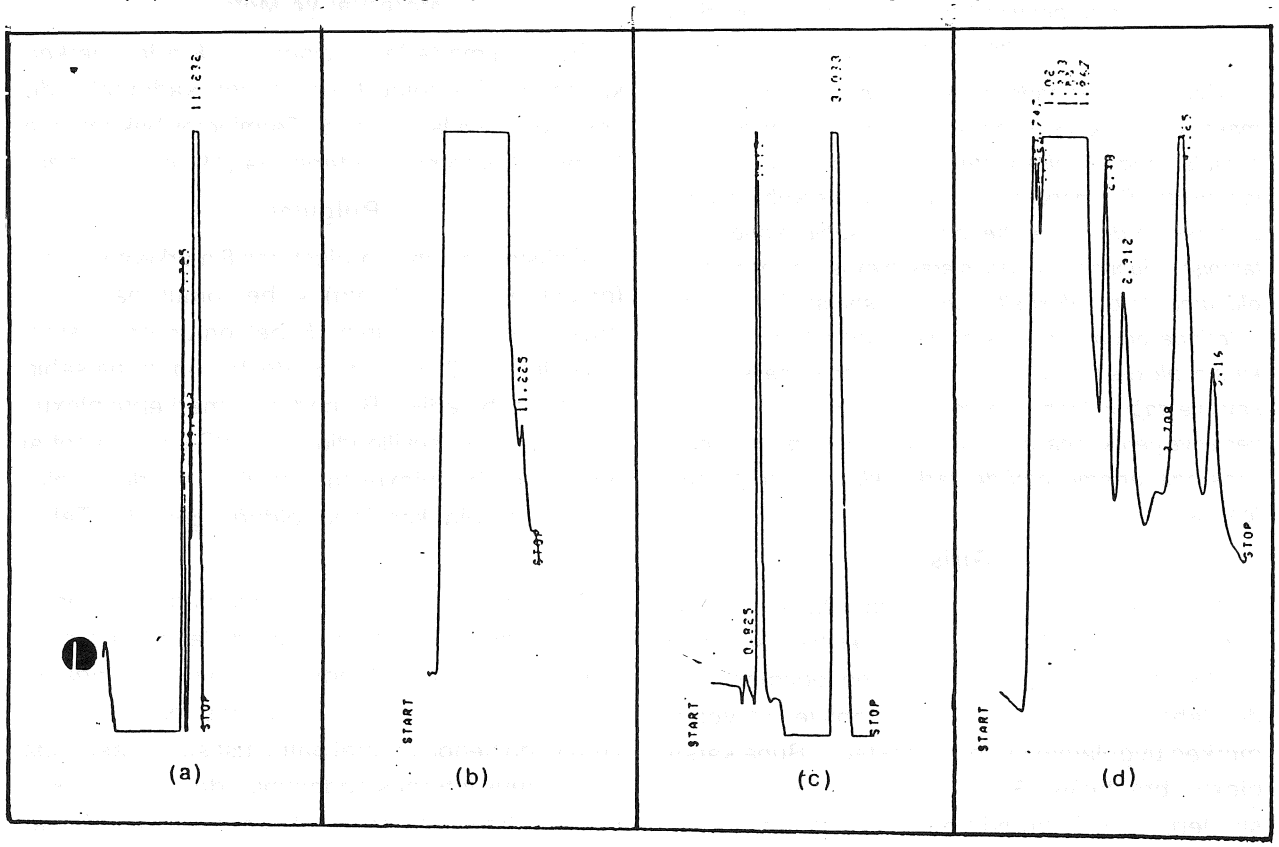

Figure 4. Chromotograms of Riboflavin and Niacin
a) The standart solution of riboflavin (500 ng)
b) The plasma samples of riboflavin
c) The standart solution of niacin (50 ng)
d) The plasma samples of niacin