

EFFECT of SIMPLE INDIGESTION and PNEUMONIA on PLASMA THIAMINE CONCENTRATION in CATTLE

Sığırlarda Basit İndigesyon ve Pnömoninin Plazma Tiamin HCl Konsantrasyonu Üzerine Etkisi

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SUMMARY

This study was carried out to determine alteration of plasma thiamine concentration in ten adult cattle with pneumonia (Experimental group I) and ten adult cattle with simple indigestion (Experimental group II). Ten healthy cattle were used as a control (Control group). The experimental group I cattle showed anorexia, depression, increased respiration rate, muco-purulent nasal discharges, coughing, increased bronchio alveolar sounds, impaired ruminal motility. The most pronounced clinical findings in experimental group II cattle were anorexia, depression, no rumination and eructation and decreased reticulo-ruminal movements. The counts of bacteria and protozoa of ruminal fluid in both experimental group I and II significantly decreased ($p<0.001$). Plasma thiamine HCl concentrations were also found to be significantly reduced in both experimental groups of cattle. The mean plasma thiamine HCl concentrations in healthy cattle was 1.70 µg/ml, while the plasma thiamine HCl concentrations in experimental groups I and II were found to be 1.00 µg/ml and 0.50 µg/dl, respectively. The result of the study showed that thiamine supplementation is to be consider as part of the treatment of simple indigestion and pneumonia.

Key Words: Pneumonia, Simple indigestion, Plasma thiamine HCl, Cattle.

ÖZET

Bu çalışma 10 adet pnömoni (Deney grubu I) ve 10 adet basit indigesyonlu (Deney grubu II) sığırlarda plazma tiamin HCl konsantrasyonlarındaki değişiklikleri belirlemek amacıyla yapıldı. 10 adet sağlıklı sığır kontrol grubu olarak kullanıldı. Deney grubu I'deki hayvanlarda klinik olarak anoreksi, depresyon, solunum sayısında artış, muko-purulent burun akıntısı, öksürük, bronko-alveolar seslerde artış ve ruminal hareketlerde azalma gözlemlendi.

Deney grubu II'deki hayvanlarda en önemli klinik semptomlar anoreksi, depresyon, ruminasyon ve ruktusun olmaması ve retikulo-ruminal hareketlerde azalma idi. Her iki gruptaki sığırların ruminal sıvı bakteri ve protozoa sayısı önemli derecede azalmıştı ($p<0.001$). Bununla birlikte plazma tiamin HCl konsantrasyonu da her iki deneme grubundaki sığırlarda önemli derecede düşük bulundu. Deney grubu I ve II'de plazma tiamin HCl konsantrasyonları sırasıyla 1.00 µg/ml ve 0.50 µg/ml iken, sağlıklı (Kontrol grubu) sığırlarda ortalama plazma tiamin HCl konsantrasyonu 1.70 µg/ml idi. Bu çalışma sonucunda, pnömoni ve basit indigesyon olan sığırlarda tiamin verilmesinin tedavinin bir parçası olarak düşünülmesi gerektiği ortaya çıkmıştır.

Anahtar Sözcükler: Pnömoni, Basit indigesyon, Plazma tiamin HCl, Sığır.

INTRODUCTION

It is well known that the microorganisms in the rumen synthesize B vitamins (1). 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 (2,3). However it is now known that under certain conditions, cattle and sheep do respond to extra dietary B vitamins. Diseases affecting metabolic and digestive efficiencies are the most common factors causing B vitamins requirements (4,5).

Thiamine deficiency occurs in ruminants when ruminal synthesis decreases or when thiamine is hydrolysed by thiaminase in the rumen (6).

Simple indigestion is the most common sequela of an abrupt change in animals ration. Forages, silages and concentrates that have spoiled, molded, soured or been exposed to extreme heat or cold are usually the cause of simple indigestion. Abrupt changes in feed present ruminal microflora with feed substrates to which they are not metabolically adapted or to which they are less adapted. Generally, the

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disease is mild and self-limiting. Changes in ruminal fluid pH are mild, and may tend toward either acidosis or alkalosis. An analysis of the ruminal fluid will show inactivity of the microflora. Ruminal motor activity in these cases is reduced, but usually not absent (7).

The pneumonia is the most common lower respiratory disease of ruminants which is characterised by invasion of pathogenic organisms, septic embolization of the lungs and interstitial reaction resulting anorexia, increased respiration rate, abnormal respiratory sounds, coughing and impaired ruminal activity (8). Unfortunately no chemical data have been reported on the alteration of blood thiamine concentration in pneumonia.

The objective of this study was to determine alteration of plasma thiamine concentration in cattle with simple indigestion and pneumonia, and to provide unique insight on the supplementation of this vitamin in the treatment of the these diseases.

MATERIALS and METHODS

Animals: In this study, ten adult cattle with pneumonia (Experimental group I) and ten adult cattle with simple indigestion (Experimental group II) which were referred to the clinic of Internal Medicine, Faculty of Veterinary Medicine, and ten clinically healthy adult cattle (Control group) belonging to Central Animal Research Institute were used as a material. The diagnosis was made according to the anamnesis, clinical and laboratory findings.

Clinical examinations: All animals were examined with regard to appetite, general appearance and behaviour, rectal temperature, rumination, eructation, reticulo-ruminal movements, rectal examination, nasal discharge, respiratory sounds and percussion of thorax.

Laboratory examinations: Ruminal fluid samples for the determination of color, consistency, odour, ruminal fluid pH, microscopic examination of protozoa, methylene blue reduction time, and protozoa and bacteria counting and venous blood samples (heparinised) for the determination of plasma thiamine HCL concentrations were collected from each animal af-

ter clinical examinations. Ruminal fluid samples were taken by passing a stomach tube and analysis were performed immediately after collection.

Rumen fluid pH determination was measured by means of electronic pH meter (ACT pH meter, Piccola Model Singapore). Microscopic examination of protozoa was performed as the method described by Turgut K (9), and the numbers of motile protozoa in a microscope area; 21-30, 11-20, 1-10 and no protozoa were represented as 4, 3, 2 and 1, respectively. Methylene blue reduction time was determined as the method described by Turgut K (9). The lost of blue color among 1-5, 6-10, 11-15 minute or more time in this tests were represented as 4, 3, 2 and 1, respectively.

The count of the protozoa and bacteria of ruminal fluid samples were determined as the method described by Eksen et al (10).

The plasma samples were stored at -25 °C deepfreeze until tested and plasma thiamine HCL concentration was measured by HPLC method described by Olkowsky et al (11).

Statistical analysis: Statistical significance were tested using Kruskal-Wallis test between Control and Experimental groups for ruminal fluid pH, microscopic counts of motile protozoa, methylene blue reduction time, the counts of the protozoa and bacteria of ruminal fluid and plasma thiamine HCL concentration. Correlation were performed among the ruminal pH, the counts of bacteria and protozoa of ruminal fluid and the plasma thiamine HCL concentration (12).

RESULTS

All routine clinical examination findings and the laboratory results were normal and between normal range in control group of cattle.

The experimental group I cattle showed anorexia, depression, increased respiration rate, muco-purulent nasal discharge, coughing, increased broncho alveolar sounds, impaired ruminal motility. Three cattle in this group had increased rectal temperature (>39.5 °C). The most pronounced clinical findings in experimental

group II cattle were anorexia, depression, no rumination and eructation and decreased reticulo-ruminal movements

The color, consistency and odor of ruminal fluid samples in experimental group I and II were clear yellow, very viscous and watery, and lost of the normal aromatic odor.

The mean ruminal fluid pH, the microscopic counts of motile protozoa, methylene blue reduction time, the counts of bacteria and protozoa of ruminal fluid samples and the plasma thiamine HCL concentrations in all groups and

their statistical significance are given in Table I.

It was found that the counts of bacteria and protozoa of ruminal fluid in both experimental group I and II significantly decreased ($p<0.001$). Parallel to this decrements, plasma thiamine HCL concentrations were also found to be significantly reduced in both experimental groups of cattle.

The result of correlation showed that there were positive correlation among the plazma HCL concentration and the counts of bacteria and protozoa of ruminal fluid (Table 2).

Table 1. The median ruminal fluid pH, the microscopic counts of motile protozoa, the methylene blue reduction time, the counts of bacteria and protozoa of ruminal fluid samples and the plasma thiamine HCL concentrations in all groups and their statistical significance.

Tablo 1. Bütün gruplarda ruminal sıvının pH'sı, hareketli protozoonların mikroskopik sayımı, metilen blue indirgenme zamanı, ruminal sıvı örneklerinin bakteri ve protozoon sayısı ve plazma tiamin HCL konsantrasyonlarının median'ları ve bunların istatistiksel önemlilikleri.

PARAMETERS	G R O U P S		
	Control Group n:10	Experimental Group I n:10	Experimental Group II n:10
pH of ruminal fluid	7.0	6.7	6.5*
The microscopic counts of motile protozoa	4	3**	2**
Methylene blue reduction time (minute)	4	3**	1**
Bacterial counts of ruminal fluid ($\times 10^9$ /ml)	9.00	6.54**	7.27**
Protozoa counts of ruminal fluid/ml	616.67	408.33**	33.33**
Plasma thiamine HCL (μ g/ml)	1.70	1.00**	0.50**

*: $p<0.05$ **: $p<0.001$

Table 2. The results of correlation among the ruminal pH, the counts of bacteria and protozoa of ruminal fluid and the plasma HCL concentration.

Tablo 2. Ruminal sıvı pH'sı, ruminal sıvının bakteri ve protozoon sayısı ve plazma tiamin HCL konsantrasyonları arasındaki korelasyonun sonuçları.

PARAMETERS	pH of ruminal fluid	Bacterial counts of ruminal fluid	Protozoa counts of ruminal fluid
Bacterial counts of ruminal fluid	0.204		
Protozoal counts of ruminal fluid	0.598**	0.372*	
Plasma thiamine HCL	0.317**	0.271*	0.422**

*: $p<0.001$ **: $p<0.01$

DISCUSSION

In this study, drastical decreases in the plasma thiamine HCl concentration ($p < 0.001$) were observed in both cattle with simple indigestion and with pneumonia. The mean plasma thiamine HCl concentration in healthy cattle (control group) was 1.70 µg/ml, while the plasma thiamine HCl concentration in experimental group I and II were respectively found to be 1.00 µg/ml and 0.50 µg/ml, respectively.

The ruminal environment and its resultant microbial population are controlled by a variety of factors. The factors related to the animal include salivation, mixing and rumination, removal of fermentation products by absorption, ingesta out flow through the reticulo-omasal orifice and eructation. Fermentative problems such as simple indigestion typically arise from abnormalities in the feed. Pneumonia which is the most common lower respiratory problem; on the other hand, may result in disruption of the neuromuscular impulses controlling the ruminal contraction and may cause decreased microbial activity (7). So, as seen in the result of this study both simple indigestion and pneumonia caused decreased microbial activity of rumen because of the anorexia, impaired ruminal motility, decrements in the counts of bacteria and protozoa in ruminal fluid, and increased methylene blue reduction time.

There is a net synthesis of vitamin B complex in the rumen (3,13) and it is commonly accepted that the rumen bacteria play a great role in this synthesis (10,13,14). Some research workers have stated that the protozoa in the rumen also took part into this synthesis (5,15). According to the result of this study, decrements of plasma thiamine concentrations in both experimental groups could be both the lack of the sufficient number of protozoa and decrease or change of the bacterial flora of the rumen. This result can be supported with the positive correlation between plasma thiamine HCL concentrations and the counts of bacteria and protozoa in ruminal fluid.

Thiamine plays two major roles in the body. It is a co-factor of enzymes that are involved in the intermediate metabolisms in the decarboxylation of alpha-keto acids and is also a

co-enzyme of transketolase. Secondly, thiamine is believed to be an active agent in the nervous system, participating in the excitation of peripheral nerves (16).

Stress or diseases affecting metabolic and digestive efficiencies are the most important factors affecting vitamin requirements in ruminants (16). Concentrations of vitamin B complex in the ruminal fluid represent a balance between additions through feed intake and microbial synthesis and removal by absorption from the rumen, passage to the abomasum, utilisation by microorganisms and possible chemical or microbial inactivation (17). So, decrements in the counts of bacteria and protozoa in ruminal fluid, impaired absorption and increased metabolic demand for thiamine in the absence of increased supply associated with increased urinary excretion of thiamine may cause the decrease in plasma thiamine concentration. The more drastical decrement in the plasma thiamine HCL concentration in the experimental group II compare to that of experimental group I could be explained by the more severe decrements or changes in the of bacterial flora and the counts of protozoa of ruminal fluid.

In conclusion, the result of the present study showed that thiamine supplementation is to be consider as a part of the treatment of simple indigestion and pneumonia and supplementation of this vitamin will help to prevent the subsequent deficiencies.

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