

THE RELATIONSHIPS BETWEEN RUMINAL JUICE, URINE, SERUM AND FECAL ZINC IN EXPERIMENTALLY ZINC DEFICIENT OSSIMI LAMBS

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ABSTRACT

This study was conducted to evaluate the relationships between ruminal juice, urine, serum and fecal Zinc (Zn) concentrations in experimentally Zn deficient lambs. Fifteen lambs with average age 5-6 months and body weight 28-30 kg were divided into two groups, first group (10 lambs) for induction of Zn deficiency and the second group (5 lambs) as control one. Ruminal juice, urine, serum and fecal samples were collected every two weeks until 12 weeks for measuring of Zn. The results revealed that alopecia, skin abnormalities, loss of appetite and emaciation were shown in experimentally induced Zn deficient lambs from the 6th week. Serum, ruminal juice, fecal and urine Zn concentrations were significantly decreased ($p < 0.05$) in the experimentally induced Zn deficient lambs from the 8th, 10th, 6th and 8th week respectively than that of the control one. Significant ($p < 0.05$) decrease in ALP and SOD were detected in Zn deficient lambs than that of control one. Significant ($p < 0.05-0.01$) positive correlations between serum Zn and urine, ruminal juice and fecal Zn were detected. There was significant positive correlation between ruminal juice and urine Zn. There was significant ($p < 0.01$) positive correlation between urine and fecal Zn. Evaluation of Zn concentrations in different body fluids rather than blood serum can be used as diagnostic tool for diagnosis of Zn deficiency in lambs. Fecal Zn concentration can be used as a biomarker for early diagnosis of Zn deficiency in lambs.

Key words: Zinc, deficiency, Relationships, Lambs

INTRODUCTION

Zinc (Zn) is an essential trace element and many physiological processes are impaired if it is not supplied in sufficient quantities in the diet. Zn is a component of many metalloenzyme such as copper-Zn superoxide dismutase (Cu-Zn SOD), carbonic anhydrase, alcohol dehydrogenase, carboxypeptidase, alkaline phosphatase and RNA polymerase, which affect on the metabolism of carbohydrate, proteins, lipids and nucleic acid, also Zn has an influence on immune system (NRC, 1985; Shankar and Prasad, 1998) as well as is a component of thymulin (a hormone produced by thymic cells that regulates cell-mediated immunity). The animals deficient in Zn exhibit atrophy of the thymolymphatic system, depressed cell-mediated immunity and increased to infection. Secondary Zn deficiency may be caused by several factors such as the consumption of immature grasses which affects digestibility, the feeding of late-cut hay which may be poorly digestible and the

presence of excessive dietary sulfur (Radostits *et al.*, 2000). High Ca content of the diet raised the Zn requirement and reduces Zn absorption (Miller, 1967). Plasma, urinary, and hair Zn are reliable biomarkers of Zn status (Lowe *et al.*, 2009). In this study, we try to use body fluids rather than blood such as, feces, urine and ruminal juice and their relationships for determination of its Zn contents as a trial for early diagnosis of Zn deficiency in sheep.

MATERIALS AND METHODS

Fifteen female, six-months-old Ossimi lambs with an average live weight of 30.45 ± 1.2 kg. kg in a private farm in Kaliobia governorate were used. Lambs were classified into two groups, the first group as control one (N=5) and the second group for experimental induction of Zn deficiency (N=10). Fecal examination, liver function, and kidney function tests were carried out for detection of any internal parasite or any liver and kidney affections that occur for animals after exposure to systemic anthelmintic (Ivermectine + clorsulon 0.2 mg / kg body weight s/c) 2 doses by 2 weeks intervals before induction of Zn deficiency. Zn deficiency was induced by

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feeding of lambs on high calcium corn soya bean meal diet by increasing the calcium and phosphorus level in the ration (Miller, 1967) by addition of ground limestone and steamed bone meal. Composition and analysis of the experimental and control ration as shown in table (1). Distilled water was offered *ad libitum*.

Blood samples were collected from jugular vein. The samples were allowed to clot in slanting position at room temperature for about 2 hours then the samples were centrifuged at 3000 rpm for 10 minutes, the clear sera were aspirated carefully by automatic pipette and transferred into clear dry labeled Eppendorf tubes and stored at -20°C till analysis. Only clear non-hemolyzed sera were used for the biochemical determination of Zn, alkaline phosphatase (AP) and superoxide dismutase (SOD).

Serum, fecal, urine and ruminal juice samples were collected at 2 weeks intervals until clinical signs of Zn deficiency were appeared.

Zn levels were detected by using Atomic Absorption Spectrophotometer (AAS) according to Fuwa *et al.* (1964). AP was measured by using of the special kits according to REC. GSCC (DGKC) (1972) while SOD was measured by method according to Nishikimi *et al.* (1972).

STATISTICAL ANALYSIS

The obtained results from the experiments were expressed as mean \pm SEM and were analyzed by analysis of variance (ANOVA) for repeated measures with means tested for significance by Duncan's

multiple range tests. The correlations between the obtained results were tested with a Pearson correlation test. Differences were declared significant at $P < 0.05$. SAS (2005) software was used to conduct this analysis.

RESULTS

Clinical parameters:

Comparing with control lambs, experimentally Zn deficient lambs showed paleness of mucus membranes, decreased ruminal movements, as well as increased respiratory and pulse rates from the 6th. week of the experiment. Loss of appetite, emaciation, alopecia and wool loss in different body regions of experimentally Zn deficient lambs. Skin abnormalities (rough skin, thickened, cracked, wrinkled with dandruff) were also observed.

Biochemical parameters:

There were significant ($p < 0.05$) decrease in Zn concentrations in the feces, serum, urine and ruminal juice samples from the 6th., 8th., 8th., and 10th. weeks respectively after inductions of Zn deficiency than that of control lambs (table 2). AP and superoxide dismutase were significantly decreased from the 10th. week in Zn deficient lambs than that of control ones (table 3).

There were significant positive correlations between serum Zn and urine ($p < 0.05$), ruminal juice ($p < 0.01$) and fecal Zn ($p < 0.01$). There was significant ($p < 0.01$) positive correlation between ruminal juice and urine Zn as well as there was significant ($p < 0.05$) positive correlation between urine and fecal Zn (table 4).

Table 1: Dietary supplementation and composition of control and Zn deficient lambs (kg/head/day)

Elements (kg/head/day)			Composition				
Alfa alfa hay	Protein supplement	Grains	Ca	P	CP	TDN	Zn
0.2	0.25	0.52	Control 0.84%	Control 0.45%	17%	73%	35 ppm
			Zn deficient 1.7%	Zn deficient 0.9%			

Table 2: Serum, urine, fecal, and ruminal juice zinc (ppm) levels in control and experimentally induced Zn deficient lambs.

Time	Serum		Urine		Feces		Ruminal juice	
	Control	Zn deficient lambs	Control	Zn deficient lambs	Control	Zn deficient lambs	Control	Zn deficient lambs
0- days	2.39 ± 0.04	2.39 ± 0.06	0.28 ± 0.01	0.29 ± 0.005	0.57 ± 0.04	0.60 ± 0.03	1.80 ± 0.17	1.71 ± 0.21
2 weeks	2.33 ± 0.03	2.30 ± 0.06	0.29 ± 0.001	0.27 ± 0.009	0.55 ± 0.02	0.57 ± 0.02	1.81 ± 0.27	1.56 ± 0.24
4 weeks	2.42 ± 0.01	2.40 ± 0.08	0.25 ± 0.002	0.26 ± 0.004	0.43 ± 0.02	0.48 ± 0.02	1.69 ± 0.1	1.62 ± 0.11
6 weeks	2.34 ± 0.04	2.16 ± 0.1	0.28 ± 0.01	0.24 ± 0.005	0.49 ± 0.02	0.31 ± 0.03*	1.36 ± 0.1	1.39 ± 0.13
8 weeks	2.35 ± 0.06	1.87 ± 0.2*	0.25 ± 0.001	0.17 ± 0.009*	0.44 ± 0.06	0.26 ± 0.02**	1.16 ± 0.1	1.07 ± 0.11
10 weeks	2.51 ± 0.05	1.43 ± 0.07*	0.27 ± 0.02	0.17 ± 0.007*	0.54 ± 0.03	0.21 ± 0.01**	1.26 ± 0.1	0.77 ± 0.12*
12 weeks	2.41 ± 0.05	1.09 ± 0.04**	0.28 ± 0.02	0.13 ± 0.007**	0.55 ± 0.01	0.18 ± 0.01**	1.40 ± 0.16	0.64 ± 0.14*

* $P < 0.5$, ** $P < 0.01$ **Table 3:** AP and SOD levels in control and experimentally Zn deficient lambs.

Time	AP		SOD	
	Control	Zn deficient lambs	Control	Zn deficient lambs
0- days	151.1 ± 11.1	146.1 ± 10.2	23.4 ± 0.3	24.4 ± 0.7
2 weeks	148.4 ± 8	132.7 ± 9	24.1 ± 0.4	22.2 ± 0.4
4 weeks	130.9 ± 6.9	128.1 ± 9.1	19.8 ± 0.5	20.1 ± 0.6
6 weeks	135.2 ± 7.3	131.5 ± 6	22 ± 0.7	19.6 ± 0.7
8 weeks	141.3 ± 5.9	136.2 ± 8	20.7 ± 0.9	19 ± 0.6
10 weeks	136.1 ± 8.6	91.7 ± 4.3*	19.5 ± 1.1	16.5 ± 0.5*
12 weeks	132.2 ± 6.2	65.3 ± 3.6**	22.8 ± 0.5	14 ± 0.8**

* $P < 0.5$, ** $P < 0.01$ **Table 4:** Correlations (r) between Zn concentrations in urine, serum, feces and ruminal juice in Zn deficient lambs.

	Urine	Serum	Feces	Ruminal juice
Urine	-	0.90**	0.63*	0.80**
Serum	0.90**	-	0.74**	0.96**
Feces	0.63*	0.74**	-	0.84**
Ruminal juice	0.80**	0.80*	0.84**	-

* $P < 0.05$, ** $P < 0.01$

DISCUSSION

Zn is an essential trace element that is required by all cells in animals as well it plays a clear and effective roles in numerous enzymatic reactions, nevertheless deficiency of Zn are associated with reduced growth rate, poor immune function, decrease reproductive performance, as well as causing skin abnormalities. Decrease of appetite in Zn deficient lambs which represented by significant decrease in the ruminal movement may due to reduced ability to taste and smell foods (Droke *et al.*, 1993a) whereby changes in appetite are associated with changes in the concentration of amino acid derived neurotransmitters in the brain, thus some trace elements deficiency as Zn may reduce the appetite by impairing the taste because it postulated that the sense of taste is mediated through the salivary Zn dependent therefore low salivary Zn concentration leads to a reduction of taste and reduced appetite. (Failla, 2003).

Reduced appetite has been also reported in buffalo calves affected with Zn deficiency (Al-Saad *et al.*, 2006). As well as reduced appetite and ruminal movement can postulate the reduction in the body weight (Van Wouwe, 1989) and body weight gain in experimentally Zn deficient sheep in this study.

Alopecia was the second most frequent sign in sheep with Zn deficiency. This finding is in accordance with those of others in calves (Machen *et al.* (1996); Radostits *et al.* (2000); Sharma and Joshi (2005)) and buffalo calves (Al-Saad *et al.*, 2006). Of all tissues, the skin has the third highest abundance of Zn in the body. In the skin, the Zn concentration is higher in the epidermis than in the dermis, owing to a Zn requirement for the active proliferation and differentiation of epidermal keratinocytes (Ogawa *et al.*, 2016).

The respiratory and heart rates were significantly higher ($p < 0.05$) in Zn deficient sheep than in normal control sheep. These could be due to the fact that Zn is a component of the enzyme carbonic anhydrase, which is located in the red blood cells and parietal cells of the stomach and is related to the transport of respiratory carbon dioxide and the secretion of hydrochloric acid by the gastric mucosa (Radostits *et al.*, 2000) as well as carbonic anhydrases are metalloenzymes that catalyze the reversible interconversion of CO_2 and HCO_3^- (Aggarwal *et al.*, 2015), so Zn deficiency causing disturbances in carbonic anhydrases which can no longer perform the CO_2 to HCO_3^- (Kimber and Pai, 2000) resulting in accumulation of carbonic acid and carbon dioxide which manifested clinically in the form of increased respiratory and heart rate.

Plasma Zn which represents, 0.2% of total body Zn content, was the most frequently measured biomarker of Zn status, thus enabling the most comprehensive

analysis of this biomarker (Lowe *et al.*, 2009). Although plasma Zn concentration responds to altered intake over short periods, the homeostatic mechanisms that act to maintain plasma Zn concentration within the physiologic range (namely, adaptive changes in efficiency of absorption and levels of endogenous excretion) may prevent high plasma concentrations from being sustained over a prolonged period (Lowe *et al.*, 2009).

Plasma Zn concentration can fall in response to factors unrelated to Zn status or dietary Zn intake, including infection, inflammation, stress, or trauma. Conversely, tissue catabolism during starvation can release Zn into the circulation, causing a transient increase in circulating Zn levels (Hambidge *et al.*, 1989). The reliability of plasma Zn concentration as a biomarker of Zn status is also dependent on the proper collection and storage of the sample, because adventitious Zn can easily be added to samples by environmental exposure and inappropriate handling of samples. Care must be taken to avoid contamination from the collection or storage vessel, hemolysis of the sample when Zn is released from the red blood cells into the plasma. The time between taking the sample and the separation of the plasma from the red blood cells can also be crucial (Lowe *et al.*, 1998). Plasma Zn levels are not considered an accurate reflection of dietary Zn intake or status (Wood, 2000). Plasma Zn content is generally considered a poor measure of marginal Zn deficiency (King, 1990) while, urinary Zn excretion can provide useful information on Zn status in Zn-supplemented individuals, but whether these reflect Zn status in depleted individuals is not certain. It is clear that there is an urgent need to develop new biomarkers of Zn status (Hooper *et al.*, 2009). A significant decrease in urine Zn concentrations in this study is agreed with the results obtained by Wood (2000).

A significant positive correlation between plasma and urine Zn may indicate that inefficiently transportation of Zn to the tissues and that some of Zn is excreted in the form of small molecular weight collates in to the urine (Main *et al.*, 1982) and this is postulated by Van Rij *et al.* (1979) who found that intravenous injection of Zn caused significant increase in urine Zn excretion by six folds, as well as Zn supplementations significantly increased serum Zn (Bicer *et al.*, 2011) urinary excretion of Zn (Eskici *et al.*, 2016).

The major change in obligatory Zn losses in response to various dietary Zn loads is achieved by altering endogenous fecal Zn losses (King and Keen, 1994). Endogenous fecal Zn is a major regulatory focal point of whole body Zn homeostasis (Wood, 2000) A significant positive correlation between plasma and fecal Zn this may be attributed to that Zn losses principally in feces (Pond *et al.*, 1995) and this confirmed the results of our study where there was a

significant positive correlations between plasma and fecal Zn as well as significant decrease in fecal Zn concentrations in experimentally Zn deficient lambs earlier than that of serum, urine and ruminal juice. So significant decrease in the fecal Zn in this study may indicate that absorption of Zn into the bloodstream does not occur from the ruminant fore-stomach; however, Zn uptake occurs in ruminal tissue (Wright *et al.*, 2008). A significant decrease in the ruminal juice Zn concentrations in Zn deficient sheep may indicate that Zn was uptake by the ruminal tissue and it was not absorbed from the ruminant fore-stomach to the blood stream (Wright *et al.*, 2008).

Because Zn has an important role in many enzymes whether changes in some of these enzyme activities might be a biomarker of Zn status (Prasad *et al.*, 1978). AP is a Zn dependent enzyme and dietary Zn deficiency will impair its activity (Vergnes *et al.*, 1992) Plasma AP appeared to show as possible Zn-dependent biomarkers (Baer and King, 1984; Kawamura, 2016). Significant decrease in AP in this study with significant decrease in plasma Zn postulated that positive correlations between Zn and AP and this results were confirmed by (Naitana, 1984) as well as Nagalakshmi *et al.* (2009) who found that significant increase in the AP in Zn supplemented lambs.

SOD enzymes catalyze the dismutation of superoxide radical into hydrogen peroxide (H₂O₂) and molecular oxygen (O₂) and consequently present an important defense mechanism against superoxide radical toxicity (Assady *et al.*, 2011; Kim *et al.*, 2000) and it contains Cu\ Zn at its active site (Thomas, 2006). In this study significant decrease in SOD in Zn deficient lambs and this results are not agree with the results obtained from Esen Gursel and Tekeli (2009) who found that Zn deficiency increase SOD in rats due to Zn deficiency resulted in increased lipid peroxidation and consequently increased SOD as a compensatory action against excessive lipid peroxidation, while Aggarwal *et al.* (2015) concluded that dietary Zn oxide supplementation increased total SOD and improved the antioxidant capacity. As well as Paik *et al.* (1999) postulated that extracellular SOD activities are decreased in subjects with low serum Zn concentrations and suggest that extracellular SOD activity may be a functional indicator of Zn nutritional status.

CONCLUSION

From the obtained results of this study, we can conclude that, evaluation of Zn concentrations in different body fluids rather than blood serum can be used as diagnostic tool for diagnosis of Zn deficiency in lambs. Fecal Zn concentration can be used as a biomarker for early diagnosis of Zn deficiency in lambs.

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علاقة تركيز الزنك في عصارة الكرش والبول ومصل الدم والبراز في الحملان الاوسيمي المصابه بنقص الزنك التجريبي

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اجريت هذه الدراسه علي عدد خمسة عشر من الحملان الاوسيمي متوسط اعمارهم من ٥-٦ أشهر وتتراوح أوزانهم بين ٢٨-٣٠ كيلوجرام وذلك لتقييم علاقة تركيز الزنك في عصارة الكرش والبول ومصل الدم والبراز في الحملان الاوسيمي المصابه لنقص الزنك التجريبي. تم تقسيم الحيوانات الي مجموعتين الأولى تتكون من عدد عشر حيوانات تم احداث نقص الزنك التجريبي بها في حين المجموعه الثانيه كمجموعه ضابطه تتكون من عدد خمس حيوانات. تم تجميع عينات عصارة الكرش ومصل الدم والبول والبراز كل اسبوعين من بداية التجربه حتي اثني عشر اسبوعا لقياس الزنك. اظهرت النتائج ظهور أعراض اكلينيكيه لنقص الزنك مثل تغيرات جلديه وتساقط الصرغ وفقدان في الشهيه وهزال في الاغنام التي تعرضت لنقص الزنك التجريبي بداية من الاسبوع السادس من التجربه. كما اظهرت النتائج وجود نقص معنوي في تركيز الزنك في مصل الدم وعصارة الكرش والبراز والبول بدا من الاسبوع الثامن والعاشر والسادس والثامن علي التوالي بالمقارنه بالمجموعه الضابطه. مع وجود نقص معنوي في مستويات ALP وكذلك SOD في الحملان التي تعرضت لنقص الزنك التجريبي عن المجموعه الضابطه كما أظهرت النتائج أيضا وجود ارتباط معنوي موجب بين تركيز الزنك في مصل الدم مع تركيزه في كلا من البول وعصارة الكرش والبراز كما ان هناك ارتباط معنوي موجب في مستويات الزنك بين كلا من البول والبراز. بناء علي نتائج هذه الدراسه نخلص أن تقييم مستويات الزنك في السوائل الحيويه المختلفه فضلا عن مصل الدم يعتبر وسيله لتشخيص نقص الزنك في الحملان لا سيما قياسه في البراز كدليل للتشخيص المبكر لنقص الزنك في الحملان.