

## Biochemical Alterations in Zinc Deficient Sheep Associated by Hyperlactatemia

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### ABSTRACT

Blood samples from diseased and clinically healthy Balady sheep of both sexes were collected and subjected for biochemical analysis of serum glucose, fructosamine, lactate, growth hormone, insulin, creatine phosphokinase, Lactate dehydrogenase and aldolase. The obtained results revealed a significant decrease in serum zinc and growth hormone were stated. In contrary, serum glucose and lactate, insulin, CPK, LDH and aldolase were significantly increased statically. While, fructosamine levels were non-significantly increased. From these results can conclude that, in zinc deficient sheep the carbohydrate metabolism is directed toward the anaerobic one accompanied by hyperlactatemia which induce muscle cramp and damages leading to increased CPK, LDH and aldolase which appear clinically as enlargement of knee joint, lameness and staggering gait. In addition, growth retardation and wool loss are the main clinical signs of reduction in growth hormone.

**Keywords:** Hypozincemia, Anaerobic Metabolism, Hyperlactatemia Growth Retardation

### 1. INTRODUCTION

Trace elements are very essential to lots of biomolecules such as hormones and enzymes. So, they should be incorporated in the food of human being and animals (Dillitzer *et al.*, 2011). Zinc is one of those important elements which considered a component of hundreds of enzymes and is involved in the synthesis, storage and release of insulin (Miao *et al.*, 2013). Moreover, it is essential for other hormones such as GH, thyroid stimulating hormone, Luteinizing hormone, follicular stimulating hormone and adrenocorticotrophic hormone (Alves *et al.*, 2012). Zinc an intracellular cation, is required to serve either a catalytic or structural role of many enzymes in mammals (Jeong *et al.*, 2012). Zinc-containing enzymes participate in many components of metabolism and cell replication. In addition, some zinc-containing enzymes, such as carbonic anhydrase and lactate dehydrogenase, are involved in intermediary metabolism during exercise. Another

zinc-containing enzyme, superoxide dismutase, protects against free radical damage (Kavas *et al.*, 2013). Zinc deficiency might be explained by the lack of key enzymes required for carbohydrate metabolism because of reduced expression of zinc dependent messenger RNA needed to synthesize these enzymes (Li *et al.*, 2013). So, zinc deficiency conveys the carbohydrate metabolism to word the anaerobic metabolism.

Zinc deficiency might be reduced animal's appetite by impairing the taste which depends on salivary zinc dependent polypeptide (Suzuki *et al.*, 2011). The reduced appetite in zinc deficient animals could be the reason for the paleness of mucous membranes, depression of growth rate and the lowered body weight (Al-Saad *et al.*, 2010). In the same context, zinc is crucial for normal development and function of neutrophils and natural killer cells. Moreover, zinc is important for gene regulation within the lymphocytes, T-cell activation, T-helper 1 and cytokine production, growth, reproduction, development, differentiation, antioxidant functions, gene expression, DNA synthesis,

neurotransmitters and apoptosis (Fouda *et al.*, 2011). Clinically, zinc deficiency is associated with parakeratosis, growth retardation, wrinkled skin, wool loss, swollen hocks, excessive salivation, alopecia, scaling and crusting of the skin of the face, neck and distal extremities (Al-Saad *et al.*, 2010).

Our study aimed to investigate the biochemical effects of zinc deficiency in lambs on carbohydrate metabolism and growth throughout the biochemical analysis of serum glucose, fructosamine, lactate, Growth Hormone (GH), insulin, Creatine Phosphokinase (CPK; EC 2.7.3.2), lactate dehydrogenase (LDH; EC 1.1.1.27) and aldolase (EC; 4.1.2.13).

## 2. MATERIALS AND METHODS

### 2.1. Animals and Experimental Design

The present investigation was carried out on twenty diseased Balady sheep (4-5 years old) of both sexes from a flock of sheep at Al-Beheira Governorate, Damanhour city. Another twenty clinically healthy Balady sheep related to another flock at the same locality fed on balanced concentrate mixture and roughage were served as a control group.

### 2.2. Blood Samples

The blood samples were collected from the jugular vein by using a sterile sharp needle with wide pore. Serum samples were collected in dry clean tubes and separated by centrifugation at 3000 RPM for 10 min then clear serum supernatants were aspirated carefully and subjected to biochemical analysis.

### 2.3. Biochemical Analysis

Collected serum samples were subjected to biochemical analysis of zinc, glucose, fructosamine, lactate, insulin, GH, CPK, LDH and aldolase. All biochemical parameters were analyzed by commercially available kit methods.

**Table 1.** Mean values of zinc (ug/dl), fructosamine (mmol/L), glucose (mg/dl), lactate (mg/dl), insulin (ng/ml) and GH (ng/ml) in control and diseased groups

	Zinc	Glucose	Fructosamine	Lactate	Insulin	GH
Control group	70.74±2.37 <sup>a</sup>	42.36±0.89 <sup>b</sup>	2.00±0.14 <sup>a</sup>	10.23±0.64 <sup>b</sup>	11.72±1.80 <sup>b</sup>	1.46±0.24 <sup>a</sup>
Diseased group	44.13±3.16 <sup>b</sup>	72.19±4.17 <sup>a</sup>	3.35±0.63 <sup>a</sup>	160.54±21.46 <sup>a</sup>	28.62±3.34 <sup>a</sup>	0.08±0.01 <sup>b</sup>

Values are mean ± standard error of mean; Means within the same column carrying different letters are significantly different (p<0.05)

**Table 2.** Mean values of LDH (U/L), CPK (U/L) and aldolase (U/L) in control and diseased groups

	LDH	CPK	Aldolase
Control group	349.00±7.59 <sup>b</sup>	148.16±5.43 <sup>b</sup>	2.59±0.26 <sup>b</sup>
Diseased group	2165.30±9.58 <sup>a</sup>	314.00±11.53 <sup>a</sup>	13.23±2.01 <sup>a</sup>

Values are mean ± standard error of mean; Means within the same column carrying different letters are significantly different (p<0.05)

### 2.4. Statistical Analysis

The descriptive data are presented as the means ±SE. The statistical differences were calculated based on two way test of ANOVA and p<0.05 is considered as significant between the groups.

## 3. RESULTS

The data illustrated in the diseased group in comparison to control one recorded a significant (p<0.05) decrease in the levels of serum zinc. While, the levels of serum glucose and lactate were statistically significantly increased. In regard to the fructosamine levels were increased but non-significantly. The effect of hypozincemia in diseased sheep was significantly decreased the levels of GH. In contrary, serum insulin levels were significantly increased in contribution to control one (**Table 1**).

The data presented in **Table 2** stated a significant (p<0.05) increase in the enzyme activities of CPK, LDH and aldolase in the diseased group in comparison to control one.

## 4. DISCUSSION

Careful clinical examination of diseased sheep revealed the presence of alopecia at the neck, shoulder and abdomen, easily detached wool and rough wool. Also, there was an enlargement of the knee joint. Additional signs include anorexia, depression, poor growth, pale mucous membrane, lameness, staggering gait and diarrhea were observed in zinc deficient sheep than in the apparently healthy. While, the mechanism of poor wool growths observed in hypozincemia perhaps involving impaired protein synthesis (Al-Saad *et al.*, 2010). Moreover, parakeratosis might be attributed to the importance of zinc as an activator of some enzymes contributed in carbohydrate, lipids, protein and nucleic acid metabolism (Yasuda *et al.*, 2011; Alves *et al.*, 2012).

The mechanism of alopecia in zinc deficient animal might be contributed to the reduction of the follicular epithelium capacity to produce a fiber (Finner, 2013). Alopecia was the most frequent sign in sheep with zinc deficiency. This finding is in accordance with those of others and buffalo calves (Al-Saad *et al.*, 2010).

The effects of zinc deficiency on carbohydrate metabolism in zinc deficient sheep were illustrated in **Table (1 and 2)** which represented by a significant increase in serum glucose concentrations. The same findings were reported in zinc deficient pups (Jou *et al.*, 2010) and diabetic rats (Fatmi *et al.*, 2013). The hyperglycemia associated with hypozincemia is associated with hyperinsulinemia as reported by (Suzuki *et al.*, 2011; Marreiro *et al.*, 2006; Jansen *et al.*, 2012). Hyperglycemia and hyperinsulinemia in zinc deficient lambs could be evidence that zinc deficiency decreased the insulin uptake. In the same manner, the plasma glucose of the zinc deficient group was significantly higher than control one. On the contrary, liver glycogen of the zinc deficient animals was significantly lower (Miao *et al.*, 2013).

The prolonged hyperglycemia in zinc deficient lambs were increased the glycosylated protein as fructosamine which formed due to a non-enzymatic Amadori rearrangement between glucose and amino acid residues of proteins. So, fructosamine is a medium term indicator of 2-3 weeks of hyperglycemia (Chon *et al.*, 2013). Fructosamine is also formed when the carbonyl group of glucose reacts with an amino group of a serum albumin, as the double bond to oxygen moves from the end carbon atom to the next carbon atom with the production of water (Lee *et al.*, 2013a).

In hypozincemia, hyperglycemia and hyperinsulinemia are the strict evidence of that, the carbohydrate metabolism was conveyed toward the anaerobic which produce lactate as an end product of catabolism. Plasma lactate levels in zinc deficient animals were significantly higher than those in control one (Myers *et al.*, 2012). The blood lactate concentration reflects a balance between production and uptake of lactate in tissues. Lactate is formed by reduction of pyruvate and is metabolized by oxidation to pyruvate in the reaction catalyzed by the cytosolic NAD-dependent lactate dehydrogenase (Ballas, 2013). The metabolic fate of pyruvate is mainly mitochondrial oxidation to carbon dioxide and water with accompanying energy production in the respiratory chain. The latter sequence of reactions is oxygen requiring and with insufficient oxygen supply, or if pyruvate production for other reasons exceeds the

capacity of oxidative metabolism, pyruvate will be diverted to lactate (Kruse *et al.*, 2011). In this situation, impaired cellular respiration leads to lower pH levels. Simultaneously, cells are forced to metabolize glucose anaerobically, which leads to lactate formation. Therefore, elevated lactate is indicative of tissue hypoxia, hypoperfusion and possible damage (Volpato *et al.*, 2012). The increment of lactic acid is evidenced by the duplication of LDH enzyme activities in zinc deficient lambs. Meanwhile, LDH is an essential enzyme involved in anaerobic glycolysis and is responsible for the anaerobic transformation of pyruvate to lactate (Neal *et al.*, 2013). The significant increase in LDH indicates the damage of the muscles either cardiac or striated muscle as LDH increases were accompanied by a significant increase in CPK which clinically are assayed as a marker of myocardial infarction, severe muscle breakdown and muscular damage (Tsitsimpikou *et al.*, 2013). A serum CPK activity is an indicator commonly used in the diagnosis of heart and skeletal muscle disorders (Baird *et al.*, 2012). In muscle damage, CPK levels have been increased. Such levels clearly signal strong disturbance or disintegration of striated muscle tissue with concomitant leakage of intracellular muscle constituents into the circulation (Iwasa *et al.*, 2013). Furthermore, the incidence of skeletal muscle injury is increasingly more prevalent in the preclinical evaluation of new therapeutic monitored by commonly used serological biomarkers, including CPK and aldolase (Toledano *et al.*, 2012). In addition to a significant increase in serum LDH and CPK activities a sensitive marker of myopathy aldolase was significantly increased in zinc deficient sheep. The elevated serum aldolase is associated with syndromes including myopathies with discomfort and weakness, systemic disorders and myopathology in perimysial connective tissue (Nozaki and Pestronk, 2009). Moreover, the damage of muscle tissue can be defined as the disruption of plasma membrane accompanied by the loss of muscle proteins as CPK, myoglobin, LDH, aldolase and troponin (Tsitsimpikou *et al.*, 2013). The damage in muscle fibers is a result of anaerobic metabolic pathway with accumulation of lactate which lead to muscle cramp and damage of some muscle fibers which observed clinically by enlargement of knee joint, lameness and staggering gait.

The obtained results indicated a significant decrease in growth hormone level which consequently, retards the growth of zinc deficient animal. Previous research has shown that a severe zinc deficiency can

result in impaired growth in lambs (Al-Saad *et al.*, 2010). Zinc is required for the stabilization of the DNA and RNA. In fact, all RNA polymerases (I, II, III) are zinc metalloenzymes (Lee *et al.*, 2013b). So, zinc is an essential nutrient and plays an important role in the growth. Concomitantly, zinc deficiency has been known to cause growth retardation along with a drastic decrease in GH production and/or Insulin-like Growth Factor-I (IGF-I) (Hamza *et al.*, 2012). One likely partial explanation for the growth restriction seen in zinc deficiency is the suppression of IGF-1 which the essential molecule for growth hormone physiology (Jayawardena *et al.*, 2012).

## 5. CONCLUSION

From the obtained data we can conclude that, zinc deficiency in sheep leads to hyperglycemia and hyperinsulinemia which directs the carbohydrate metabolism toward the anaerobic process which pronounced by a drastic increase in lactate which consequently accumulated in the muscles leading to muscle cramp and damages which monitored by a significant elevation of serum LDH, CPK and aldolase. Moreover, growth retardation was recorded as a result of a reduction in growth hormone.

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