

The effect of experimentally induced vitamin E and selenium deficiency on Creatine Kinase (CK) and Aspartate Aminotransferase (AST) activities in Awassi ewes and their newborn lambs

H. K. Abood A. M. H. Judi
A. A. AL-Ani

Department of Internal and preventive Vet. Med. , College of Vet. Med.,
University of Baghdad

Abstract:

Experimental induction of vitamin E and selenium deficiency by deficient diet was carried out on Awassi ewes and their newborn lambs to study the effect of the deficiency on serum levels of Creatine Kinase (CK) and Aspartate Aminotransferase (AST).

Fourteen animals in the deficient group and 7 animals in the control group were used. Results showed increased Creatine Kinase (CK) levels reaching $(2070.51 \pm 22.22 \text{ U/L})$ and $(2756.52 \pm 20.79 \text{ U/L})$ in deficient groups of ewes and lambs respectively compared with control groups in which levels reached $(211.07 \pm 2.23 \text{ U/L})$ and $(292.52 \pm 1.20 \text{ U/L})$ respectively.

Results showed increased Aspartate Aminotransferase (AST) levels reaching $(143.71 \pm 4.28 \text{ U/L})$ and $(145.40 \pm 7.94 \text{ U/L})$ in deficient groups of ewes and lambs respectively compared with control groups in which levels reached $(69.14 \pm 2.78 \text{ U/L})$ and $(72.85 \pm 2.33 \text{ U/L})$ respectively.

دراسة تأثير نقص فيتامين E والسليوم المستحدث على أنزيم ال Creatine Kinase (CK) وأنزيم Aspartate Aminotransferase (AST) في النعاج العواسية ومواليدها

حيدر كريم عبود عبد المناف حمزة
احمد علاء الدين

فرع الطب الباطني والوقائي البيطري، كلية الطب البيطري، جامعة بغداد

الخلاصة:

تم أستحداث نقص السليوم وفيتامين E باستعمال عليفة غذائية لاستحداث النقص الغذائي في النعاج العواسية ومواليدها لغرض دراسة تأثير النقص على مستوى مصل الدم من أنزيمات ال Creatine Kinase (CK) وال Aspartate Aminotransferase (AST) تم أستخدام 14 حيوان في مجموعة النقص الغذائي وسبعة حيوانات في مجموعة السيطرة.أظهرت النتائج زيادة في مستوى ال Creatine Kinase (CK) حيث وصلت المعدلات $(2070.51 \pm 22.22 \text{ U/L})$ و $(2756.52 \pm 20.79 \text{ U/L})$

20.79 U/L) في مجاميع النقص للنعاج والحملان على التوالي بالمقارنة مع مجاميع السيطرة حيث وصلت المعدلات (211.07 ± 2.23 U/L) و (292.52 ± 1.20 U/L) على التوالي. أظهرت النتائج زيادة في مستوى الـ Aspartate Aminotransferase (AST) حيث وصلت المعدلات (143.71 ± 4.28 U/L) و (145.40 ± 7.94 U/L) في مجاميع النقص للنعاج والحملان على التوالي بالمقارنة مع مجاميع السيطرة حيث وصلت المعدلات (69.14 ± 2.78 U/L) و (72.85 ± 2.33 U/L) على التوالي.

Introduction:

Creatine Kinase (CK) and Aspartate Aminotransferase (AST) levels were significantly increased in lambs affected with stiff-lamb disease and vitamin E and selenium administration was followed by significant decrease in enzymes levels (1).

Polyunsaturated fatty acids were capable of escaping ruminal hydrogenation at turnout, resulting in a three-fold increase of plasma linolenic acid within three days of turnout (2). Linolenic acid, if protected from ruminal hydrogenation, rapidly reaches high levels in blood and is associated with a rise in plasma Creatine Kinase (CK) indicating muscular degenerative myopathy (3).

An increase in serum level of Creatine Kinase (CK) in lambs suffering from nutritional muscular dystrophy was reported by (4). Creatine Kinase (CK) in plasma has been shown to be elevated in ruminants that exhibit clinical or subclinical nutritional muscular dystrophy, and is highly correlated with the degree of muscle damage in cows (5).

Levels of Creatine Kinase (CK) and Aspartate Aminotransferase (AST) were increased in calves with

white muscle disease. Vitamin E administration to calves suffered from vitamin E deficiency decreased enzymes of muscle origin such as Creatine Kinase (CK) and Aspartate Aminotransferase (AST) (6,7).

Diagnosis of white muscle disease relies on necropsy and clinical pathology especially enzymes Creatine Kinase (CK) and Aspartate Aminotransferase (AST) indicative of muscular dystrophy (8). Relevant to the diagnosis of muscular damage are Creatine Kinase and Aspartate Aminotransferase, of which Creatine Kinase (CK) is the most sensitive and specific indicator of muscular damage (9).

Prior to the first clinical signs of white muscle disease in lambs there is a certain increase in blood Creatine kinase (CK), Aspartate Aminotransferase (AST) concentrations indicative of muscle degeneration (10). Plasma Creatine Kinase (CK) is the most commonly used laboratory aid in the diagnosis of nutritional muscular dystrophy. Aspartate Aminotransferase (AST) activity is also an indicator of muscle damage. The magnitude of the increase in Aspartate Aminotransferase (AST) and Creatine Kinase (CK) is directly

proportional to the extent of muscle damage (11).

Materials and Methods:

1- Animals: Twenty one Awassi ewes and their newborn lambs from State Board of Agricultural Research / Ministry of Agriculture were used. The deficient group included 14 ewes and the control group included 7 ewes. Ultrasound scanner was used to check the uterine health of the ewes. Estrus synchronization was scheduled. The study lasted for 10 months started on 1.3.2011, ended on 1.12.2011.

2- Induction of selenium and vitamin E deficiency done by feeding a diet consisted of cod liver oil 3%, ground corn 0.5 kg/ animal, discolored bad quality hay ad lib, and water was offered ad lib (12). Feeding of this deficient diet lasted for three months (the last two months of gestation and one month after birth). The control group were allowed the regular feeding program adopted in the state board of agricultural research.

The animals in the deficient group and the control group were watched at a regular daily basis.

3- Serum Creatine Kinase (CK) was estimated according to the instructions mentioned in a kit from (Linear chemicals, S.L./ Spain) and spectrophotometer (CECEIL / England).

4- Serum Aspartate Aminotransferase (AST) was estimated according to the instructions mentioned in a kit from

(Symbio / Syria) and spectrophotometer (CECEIL / England).

5- Selenium in serum was estimated according to (13), and vitamin E in serum was estimated according to (14).

Statistical Analysis

Statistical analysis was conducted using ready – made statistical design statistical package for Windows Integrated Student Version (SPSS) (15).

Results:

Clinical signs of the deficiency appeared after three months of feeding deficient diet in ewes and the serum levels of selenium and vitamin E was 0.02 ppm, 0.61 mg/L compared with the control group 0.45 ppm and 2.72 mg/L. While the clinical signs of the deficiency in lambs appeared within three days of life and the serum selenium and vitamin E reached 0.01 ppm and 0.34 mg/L compared with the control group 0.45 ppm and 2.45 mg/L.

The results showed increased Creatine Kinase (CK) levels reaching $(2070.51 \pm 22.22 \text{ U/L})$ and $(2756.52 \pm 20.79 \text{ U/L})$ in deficient groups of ewes and lambs respectively compared with control groups in which levels reached $(211.07 \pm 2.23 \text{ U/L})$ and $(292.52 \pm 1.20 \text{ U/L})$ respectively (table 1). Results showed increased Aspartate Aminotransferase (AST) levels reaching $(143.71 \pm 4.28 \text{ U/L})$ and $(145.40 \pm 7.94 \text{ U/L})$ in deficient

groups of ewes and lambs respectively compared with control groups in which levels reached (69.14 ± 2.78 U/L) and ($72.85 \pm$

2.33 U/L) respectively (table1).Results showed significant differences between groups at $P < 0.05$.

Table (1) shows results of Creatine Kinase (CK) and Aspartate Aminotransferase (AST) activities in ewes and their newborn lambs

Parameter Group	CK (U/L)	AST (U/L)
Deficient group (Ewes)	2070.51 ± 22.22 A	143.71 ± 4.28 A
Control group (Ewes)	211.07 ± 2.23 C	69.14 ± 2.78 B
Deficient group (Lambs)	2756.52 ± 20.79 A	145.40 ± 7.94 A
Control group (Lambs)	292.52 ± 1.20 D	72.85 ± 2.33 B

Deficient group: n=14

Control group: n=7

Values represent means \pm SE

Different capital letters mean significant ($P < 0.05$) results between different group.

Discussion:

The results of this study showed that there was an increase in serum levels of Creatine Kinase (CK) and Aspartate Aminotransferase (AST) activities in selenium and vitamin E deficient ewes and their newborn lambs, this was in agreement with (1) who reported that Creatine Kinase (CK) and Aspartate Aminotransferase (AST) levels were significantly increased in lambs affected with stiff-lamb disease and vitamin E and selenium administration was followed by

significant decrease in enzymes levels.

Elevation of Aspartate Aminotransferase (AST) and Creatine Kinase (CK) in this study indicated that there was a muscle damage caused by vitamin E and selenium deficiency and these facts were supported by (10) who mentioned that prior to the first clinical signs of white muscle disease in lambs there was a certain increase in blood Creatine kinase (CK), Aspartate Aminotransferase

(AST) concentrations indicative of muscle degeneration.

The results in this study have also been supported by (1,4) who mentioned that increased serum Creatine Kinase (CK) and Aspartate Aminotransferase levels have been recorded in lambs affected by white muscle disease (WMD). This enzyme is involved in helping provide energy for muscle contraction. The duration of elevation following muscle injury is considerably less than for Aspartate Aminotransferase (AST) and the increased levels of Aspartate Aminotransferase (AST), which appears to be proportional to the degree of muscle fiber degeneration, has been used in the diagnosis of nutritional muscular dystrophy in ruminants (16,17).

References:

1-El-Neweehy, T.K.; Al-Qarawi, A.A. and Abdel-Rahman, H.A. (2000) Some studies on Stiff Lamb Disease in Qassim region in Saudi Arabia. 1: Enzymatic profile in free, subclinically and clinically affected lambs both before and after treatment with vitamin E and selenium preparation. *Small Ruminant Research* 35: 219-223.

2- McMurray,C.H.; Rice,D.A. and Blanchflower,W.J.(1980) Changes in plasma levels of linoleic acid in calves recently introduced to spring pasture.*Proc.Nutr.Soc.*39:65A (Abstr.).

3- Rice, D.A.;Blanchflower, W.J. and McMurray, C.H. (1981) Reproduction of nutritional degenerative myopathy in the post ruminant calf. *Vet. Rec.* 109:161.

4- Norton ,S. A. and McCarthy, F. D. (1986) Use of Injectable Vitamin E and Selenium-Vitamin E Emulsion in Ewes and Suckling Lambs to Prevent Nutritional Muscular Dystrophy. *J. Anim. Sci.* 62:497-508.

5- Spears, J. W.; Harvey, R. W. and Segerson E. C. (1986) Effects of Marginal Selenium Deficiency and Winter Protein Supplementation on Growth, Reproduction and Selenium Status of Beef Cattle. *J. Anim. Sci.* 1986. 63:586-594.

6-HoshinoYIchijo, S.; Osame, S. and Takahashi, E. (1989) Studies on serum Tocopherol, Selenium levels and blood Glutathione Peroxidase Activities in Calves with White Muscle Disease. *Jpn. J. Vet. Sci.* 51(4):741-748.

7-Reddy, P. G.; Morrill, J. L.; Minocha, H. C. and Stevenson, J. S. (1987) Vitamin E is immunostimulatory in calves. *J. Dairy Sci.* 70 : 993-999.

8-Bostedt, H. and Schramel, P. (1990).The importance of selenium in the prenatal and postnatal development of calves and lambs. *Biol. Trace Elem. Res.* 24 : 163-171.

- 9-Meyer, D. J. and Harvey, J. W. (1998) Veterinary laboratory medicine: interpretation and diagnosis. Second edition, W. B. Saunders Company. Pp: 373.
- 10-Chariot, P. and Bignani, O. (2003) Skeletal muscle disorders associated with selenium deficiency in humans. *Muscle Nerve*. 27: 662-668.
- 11-Radostits, O. M. Gay, C. C. ;Hinchcliff, K. W. and Constable, P. D. (2007) *Veterinary Medicine: A Textbook of the Disease of Cattle, Sheep, Pigs, Goats and Horses*, 10th ed. Saunders Elsevier, 1746.
- 12-Welch, J. G. Hoekstra, W. G.; Pope, A. L. and Philips, P. H. (1960) Effects of Feeding Fish Liver Oil, Vitamin E and Selenium to Ewes upon the Occurrence of Muscular Dystrophy in Their Lambs. *J. Anim. Sci.* 19 : 620-628.
- 13-Varley, H. Gowenlock, A. H. and Bell, M. (1976) Thetocopherols. In: Varley H (ed) *Practical clinical biochemistry*, vol. 2. Hormones, vitamins, drugs and poisons. Heinmann Medical, London, Pp: 222-223.
- 14-Norheim, G. and Haugen, A. (1986). Precise determination of selenium in tissues using automated wet digestion and automated hydride generator-atomic absorption spectroscopy system. *Acta. Pharmacology Toxicology*. 59 (7): 610 – 612.
- 15-SPSS (1996) *Statistical Packages for Windows Integrated Student Version*.Version 13.0, SPSS Inc., Chicago, Illinois.
- 16-Kumar, N. Garge, A. K. Mudgal, V.; Dass, R. S.; Chaturvedi, V. K. and Vaarshney, V. P. (2008) Effect of different levels of selenium supplementation on growth rate ,nutrient utilization,blood metabolic prophile,and immune response in lambs. *Biol. Trace Elem. Res. Suppl.* 126 :s 44-56.
- 17-Mohri, M.; Ehsani, A.; Norouzian, M. A.; Bami, M. H. and Seifi, H. A. (2011) Parenteral selenium and vitamin E supplementation to lambs:hematology,serumbiochemistr y,performance,and relationship with other trace elements. *Biol. Trace Elem. Res.* 193(3):.308-316.