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Research Article

EFFECT OF EXPERIMENTAL INDUCED HYPOCALCEMIA ON PHYSICAL PARAMETERS AND CALCIUM LEVELS IN AWASSI SHEEP

Wassan, A.G. *, Manar Hady Alawy

Dept. Internal and Preventive Medicine – College of Veterinary Medicine – Baghdad University

***Corresponding Author: Email**

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ABSTRACT

This study was induced to record the clinical signs and calcium levels in cases of induce calcium deficiency in Awassi ewes and to investigate the changes of these parameters after treatment. A study included sixty one Awassi ewes, ages ranged between 2-2.5 years and body weigh between 35-45 kg, divided to fourgroups: group one included ten ewes induce in hypocalcaemia and then treated, group two included ten ewes with clinical case: suffering from hypocalcaemia within area of Ab-Grab, group three included thirty ewes suffering from hypocalcaemia in Hours racing of Al-Amara, and group four included ten ewes considered as control group. Group one and four have a preparation period for one month. During this period they fed the same dietary material consist of concentrate and green forage, and injected Ivermectin subcutaneously and multivitamin intramuscularly and gave mineral block and drainage by Vimazole orally, then examined to insure no present of nutritional diseases, then induce hypocalcaemia in group one by injection of Na2EDTA in jugular vein, then take blood samples by different time besides recording the clinical signs. After clinical signs appear group one treated by using one of calcium compound which is Ca Gluconate monohydrate in three doses for two weeks. At the end period of treatment, blood samples was taken for this group.

Results showed a significant difference ($P<0.05$) in temperature, pulse and respiration in all groups compared with control group, as well as, the measurement of calcium levels in serum.

Keywords: sheep, hypocalcaemia, clinical signs.

INTRODUCTION

Calcium, a mineral typically found in the body, is essential for ordinary muscle and heart capacity. High calcium levels may be found in some plant inebriations, over the top dietary supplements, and a mixed bag of different conditions. Low calcium levels frequently happen in a lactating creature (milk fever), a creature in late pregnancy, or a creature with grass tetany Normal blood calcium level is between (11.9 - 12.4 mg/dl) (Kaneko,1989). Calcium has different capacities, including:

Directing skeletal, heart, and smooth muscle constriction, easing nerve motivation transmission, actuating chemicals that fortify vital body compound responses, adding to the coagulation framework, impacting cardiovascular automaticity and contractility (Ignatavicius and Workman 2010). The deficiency of calcium lead to hypocalcemia Common reasons for hypocalcaemia incorporate hypoparathyroidism, vitamin D insufficiency, and perpetual kidney disease (Armstrong and Cota, 1999). Exposure to mercury, Excessive dietary magnesium as with supplementation, Excessive diaetary zinc, as with supplementation (causes fast hypocalcemia), Prolonged utilization of drugs/diuretics containing magnesium, Chelation Therapy for metal introduction, especially EDTA, metabolic ailment of sheep, described by tetany, incoordination, and loss of motion and extreme lethargies is brought on by a deficient supply of metabolisable calcium (Jensen and Swift 1982). Many of method of hypocalcemia in ewe accure

Normal happening most generally about the time of lambing (Kott, 2005). Or Instigate Hypocalcaemia was prompted by i.v. mixtures of Na2EDTA: Hyperketonemia was kept up by i.v. implantation of DL-beta hydroxybutyrate. The tests demonstrated that induction of hypocalcaemia (Schlumbohm and Harmeyer 2003) or dose of theophylline effect (0.25mg/kg) during 40min, on levels of ca and mg inducing hypocalcaemia (Persson and Luthman 1975).

Said that (Wilson, 2001) ewes susceptibility of late-pregnant to fasting-induced hypocalcaemia when reduced in soya bean oil supplement.

Ethylene diamine tetra-acetic acid (EDTA): additional names is an aminopolycarboxylic acid and a colourless, water - soluble solid. Its conjugate base.The compound was first described in 1935 by Ferdinan Munz preparation the compound from ethylenediamine and chloroacetic acid chelate agent, i.e., its ability to "sequester" metal ions such as Fe3+ and Ca2+ (Ferdinan, 1938).

MATERIALS AND METHODS

Animals:

Sixty one Awassi ewes aged between 2-2.5 years old with body weights 35-45 kg were used in this study. Ewes were divided into four groups as following:

Group one: 10 ewes brought from abo-ghrab. This group was treated after inducing hypocalcemia.

Group two: 10 ewes' clinical case from abo-grab Regina.

Group three: 30 ewe clinical case from AL-Bakreah, AL-Amreah racing horses .

Group four: 10 of animal as control group take from field collage of veterinary medicine.

Prepare solution Na2EDTA:

According to Radostits, (2008) a weight of 5 gram from Na2EDTA put in flask then complements the size to 100ml of normal saline until the solution completely dissolved. Then the PH of solution measured then adjusted the a range of 6.8 to 7.0 with KOH . Then transfers by sterile syringe into bottle. To prevent the explosion, needle was put in the top of bottle. All these steps conducted in hood. And entry to autoclave .then cooled with water bath 50c then injected to animals.Notes/ divided the dose according to time by used canella.

Pilot study: One ewe was prepared to pilot study by take blood sample and record calcium level in serum. According to Radostits, (2008), the dose of 5% Na2EDTA is 1.2ml/kg per hour for 18hr, and this ewe has a body weight of 35 kg , and aged twenty month , so the recommended of Na2EDTA is (40ml) from prepared solution Na2EDTA. Experimentally this ewe dosed gradually as the dose 40ml divided per (2hr). After 6hr. the clinical signs appeared so the total dose was 120 ml for this ewe. During this period, blood samples were collected each 1hr to measure the serum calcium after the appearance of clinical signs.

Preparation period :(from 15/10/2014 to15/11/2014)

Including 10 ewes from Control and 10 hypocalcemia group were adapted on same diet (concentrated diet and green diet) for 30 days and injected by multivitamine to correct any deficiency in blood mineral and gave mineral block. These animals prepared for experiment by injection with ivermectin at a dose of (1ml/50kg B.W). This dose repeated again after (2 weeks), as well as the animals dosed with Vimazole 5% (East company for vet. Drugs, Syria at a dose of (1l/10kg B. w). During this period ewes examined clinically and laboratory to record weekly each of the body temperature, pulse rate, heart rate, respiration, calcium in serum during preparation period.

A. Laboratory

Inducing hypocalcemia period : from 16-11-2014 to 3-1-2015):

Ten ewes of this group was dosed as recommended Na2EDTA does (from piolet study).each ewe inducing hypocalcemia alone (i.e each day one animal). Firstly prepared animal by clipping and shaving, both jugular veins area: One of the cannulas was used for the infusion of % 5 solution of Na2 EDTA along 6 hr. the second cannula was used to collect blood samples, ECG and clinical signs was recorded, there was slightly individual variation among experimentally animals in this group this variation was :-

-Not all animals appeared the same severely clinical signs.

-Some animal have more than 6hr. to appear the clinical signs of hypocalcemia.

-Cold extremities and head deviation not appear in all animals in this group.

Control group: from16-11-2014 to 3-1-2015

During inducing hypocalcemia period there was daily recording of blood parameters and clinical signs for animals in this group. When injection of Na2EDTA in 1st group,

normal saline was injected to this group as a same dose of Na2EDTA in the1st group.in blood sample as well as to all parameters which recorded, in all animal in this group.

Treatment group: from17-11-2014 to 18-1-2015

The same 10 ewes of hypocalcemia group were considered as treated group after appearance of clinical signs, blood samples collected after that Calcium Gluconate Monohydrated was injected as 1m/animal slowly inject with monitoring the heart rate by auscultation with stethoscope. Continuous injection performed till standing up of the animal, this was the first dose , followed by two other dose during 2 week (4 days intervals) after ending of treatment period, blood sample and clinical signs were recorded.

Clinical cases : from1-12-2014 to 5-4-2015

Two groups from Abo-grap and AL-Ammeriah racing horses (40 ewes). Each ewe have a clinical signs of hypocalcemia was recorded by blood samples.

Sample collection:

Blood samples were collected from jugular vein 13 ml by vacationer apparatus about 10ml in tube without anticoagulants blood samples (gel tube). Then transported to laboratory on ice were kept for 15 minutes at room temperature and then separated by centrifugation at 3000 r.p.m for 15 min and chilled-20c until analysis of serum. Reminder of collected blood transfered by the same way in the laboratory biotechnology research center / AL-Nahrain University.

Biochemical test:

1. Estimate Serum Calcium as fallowing :

10µl serum was collected to determine the calcium according to Young, (1990).

O- cresolphthaline complexone colorimetric method:

Calcium ions react with o-cresolphthalein complexone (O-CPC) under alkaline conditions to form a violet colored complex.

Ca2+ + o-cpc alkaline pH ⟶ calcium –o-c p c complex

The color intensity of the complex formed is directly proportional to the calcium concentration. It is determined by measuring the increase in absorbance at 578nm.

Mix and incubate for minutes at 20-25 .Measure absorbance of specimen (Aspecimen) and standard (Astandard) against reagent blank. The calcium concentration in the sample is calculated using the general formula.

Procedure:

	Blank	standard	specimen
Standard		10µl	
Specimen			10µl
Reagent 1	0.5ml	0.5ml	0.5ml
Reagent 2	0.5ml	0.5ml	0.5ml

RESULT AND DISCUSSION

Pilot study:

One ewe was prepared to pilot study, calcium level in serum of this ewe was (9.5mg/dl), this ewe dosed by Na2EDTA (40ml/kg B .W) this dose dived in to 2 hr. in each hour calcium level recorded as the follow:- in 20 ml /hr. as a first dose, 8.5mg/dl calcium level recorded, and no clinical signs of hypocalcemia. The second and third dose till five dose, there is no clinical signs appear and calcium level was decline very slowly, then after six dose (reach 120ml/ kg .b .w) the clinical signs was appear (sternal recumbency ,muscle tremor ,cold extremities and turned head as S-shape and decline of calcium level reach to (4mg/dl).

Inducing hypocalcemia in this ewe of Pilot study was detected after injection of Na2EDTA according to recommended dose (Radostits, 2008), so gradually injection Na2EDTA till clinical signs of hypocalcemia appear and decided that after 6 hr. to experimental study. In each hr. blood sample was collected and calcium was recoded till after this 6 hr. reach (4 gm/dl) although, there was slightly variation in time of appearing clinical signs according to animal's susceptibility .

Inducing hypocalcaemia:

After preparing animals (Fig.1) to inducing hypocalcemia, in the group one (experimental hypocalcemia). The clinical signs show: depression, increase respiration rate, muscle tremor, muscular weakness, head twisted toward flank and sternal recumbency (Fig.2), this is the most clinical signs observed, these signs agree with (Pickard, 1987; Robinson, 1988 and Sweney and Cudderford, 1987).

This table show clinical physical examination. Experimental group showed significant difference during inducing hypocalcemia compared with all group concerning

Table 1: The clinical examination (temperature, pulse and respiration) in all groups.

(Mean±SD)		Temperature	respiratory	Pulse
Experimental	Induce	A 40.388±0.464	A 37.000±1.604	AB 88.333+7.339
	Treatment	B 39.175±0.528	B 28.000±0.756	A 84.000+4.050
Field Cases	AB-grap	C 37.450±0.607	C 18.875±1.126	AB 89.333+5.203
	racing horses	C 37.512±0.470	C 17.875±0.835	B 91.167+3.312
Control		B 39.012±0.285	B 27.125±1.553	C 75.667+ 2.338
LSD		0.468195	1.187939	4.63

Table 2: Show biochemical parameters calcium and magnesium in all groups.

(Mean± SD)		Calcium
Experimental	induce	A 4.631±2.146
	Treatment	B 8.866±0.258
Field Cases	AB-grap	C 3.964±0.455
	racing horses	C 5.666±0.313
Control		B 9.638±0.410
LSD		0.98439

temperature which show increase in temperature within normal rang compared with all group, this agree with (Radostits ,2008; Woldemeskel, et al 2000). This is due to in the first stage of hypocalcemia there is increase in temp. within normal rang, while in second and third groups they showed decrease in temp. out of normal, because the field case reached the late stage of hypocalcemia, this agree with Merck and Co, (2008(and Radostits, (2008).

In the same table there was significant increasing in respiration in group experimentally, while in filed cases second and third group there was significant decrease in respiration compared with inducing experimental group. This agree with Radostits, (2008), because in group one experimentally the hypocalcemia was in the first stage while in filed cases the hypocalcemia was in late stage. On the other hand, in group one experimentally it was shown an

increase in plus rate due to increasing of respiration and temp. in stage one of hypocalcemia while the group one experimentally treatment retained to normal range compared with group four (control) Radostits, 2008). While in group two and three there was increase in the pulse rate but decrease in respiration due to hypocalcemia that occurred since along period so stress factor and environmental condition could effect on animals activity and lead to decrease in respiration (Smith, 2009).

After treatment the experimental group in all clinical parameters showed returned to normal gradually comparative with control group .due to treatment strategies. Using three doses of calcium glucolant was better than one dose, because normal clinical signs appeared better after three doses.

After inducing hypocalcemia ,blood sample was collected to determined Ca, table (2) showed significant decrease in calcium in group one experimentally due to injection of Na2EDTA this agree with several researchers (Takagi and Block,1991 ; Fenwick and Danial,1992; Jorgensen, et al, 1999; Schlumbohm and Harmeyer, 2003). While in field cases there was significant decrease more than experimental group reach to 3.964 mg/dl compared with control group, as well as, the treatment group was rented to normal level of calcium compared with control.

REFERENCES

1. Armstron, C.M. and Cota, G.(1999). Calcium block of Na+ channels and its effect on closing rate". Proceedings of the National Academy of Sciences of the United States of America. 96, (7): 4154–4157. Bibcode: 1999PNAS. 96.4154A
2. Ferdinan, M. (1983). Polyamino carboxylic acids to I. G. Farbenindustrie, DE 718 981, 1935; US 2 130 505, 1938.
3. Fenwick, D.C and Danial, R.C.W (1992). Blood pathophysiological changes in sheep following a prolonged (18-hour) period of hypocalcaemia induce by Na2EDTA solution . British Veterinary J., 148(5):425-434.
4. Ignatavicius, D. and Workman, ML., eds (2010). Medical-surgical Nursing: Patient-centered Collaborative Care. 6th ed. St. Louis, MO: Elsevier Saunders.
5. Jensen, R. and Swift, B. L. (1982(: Disease of Sheep. 2nd ed. Lea & Febiger, Philadelphia., 19-28.
6. Jorgensen, Rj; Nyengaard, NR; Danial, RCW; Mellau, LSB; Enemark, JMD.(1999). Induced hypocalcemia by Na2EDTA infusion. A Review .J. Vet. Med. 46:389-407.
7. Kaneko, JJ. (1989). Clinical biochemistry of domestic animals, ed 4, New York, Academic Press.
8. Kott, R. (2005). Diseases and Disorders Commonly Seen in Ewes Just Prior to or Shortly After Lambing Extension Sheep Specialist, Montana State University.
9. Merck and Co (2008) .The Merck Veterinary Manual, a subsidiary of Merck and Co., Inc., Whitehouse Station, N.J., U.S.A.
10. Persson, j and luthman, j. (1975). Futher studies on theophylline-induced hypocalcaemia in sheep. Act veterinaria Scandinavica; v16(1):39-50.
11. Pickard, D.W. (1987) : Hypocalcemia in ewes. Vet. Record, 1987, 122 (4), 92.
12. Radostits, O.M.; Gay, CC.; Blood, D.C. and Hinchliff, k.w. (2008). Veterinary medicine. A textbook of the diseases of cattle, sheep, pigs, goats and horses, 10th ed .w.B. saunders company Ltd. London . New York. phliladelphia, san Francisco. Sydney.
13. Robinson, W.F (1988). Clinicopathological principles for veterinary medicine. Cambridge University Press, New York, New Rochel , 389-398.
14. Schlumbohm, C.; Harmeyer, J. (2003). Hypocalcemia reduces endogenous glucose production in hyperketonemic sheep. J. Dairy Sci.; 86(6): 1953-1962.
15. Smith,B.p.(2009). Large animal internal medicine .4th ed .Mosby Elsevier. St.louis,Missouri.
16. Sweeney, H. J.and Cuddeford, D. (1987).An outbreak of hypocalcaemia in ewes associated with dietary mismanagement. Vet. Record 120, (5):114.
17. Takagi, H. and Block, E.(1991). Effects of various dietary cation-Anion balances on response to Experimentally induced hypocalcemia in sheep .J. Dairy sci. 74:4215-4224.
18. Wilson, G-F.(2001). Stimulation of Ca absorption and reduction in susceptibility to fasting-induced hypocalcaemia in pregnant ewes fed vegetable oil.New Zealand veterinary Journal; v 49(3):115-118.
19. Woldemeskel, M.; Eneyew, M. and Kassa, T. (2000). Study on ovine hypocalcemia in ewes in central Ethiopia. Revue. Med.Vet., 151,4,345-350.
20. Young, DS.(1990). Effects of drugs on clinical laboratory tests. AACC press, Washington, D.C.

