Changes in electrocardiographic, hematologic and biochemical indices of Markhoz goat breed in experimental hypocalcemia

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Abstract:

BACKGROUND: Milk fever in cattle, sheep and goats occurs around the time of parturition and is caused by hypocalcemia. **OBJECTIVES:** The aim of this study was to investigate the physiological effects of experimental hypocalcemia on electrocardiography, hematology and serum biochemical changes in Markhoz goat breed. **METHODS:** Ethylene diamine tetra- acetate solution 4.6% was intravenously infused to 5 healthy goats (experimental group) and 5 healthy goats received 0.9% saline solution (IV) as control group. In both groups, electrocardiogram was recorded in base apex lead and serum was collected before and after infusion. Electrocardiography, biochemical and hematologic parameters were measured. Clinical signs of hypocalcaemia were caused by EDTA infusion. **RESULTS:** The results in experimental group showed a significant decrease in calcium, lactate dehydrogenase, potassium, and increase in glucose concentration, (p<0.05).The white blood cells, lymphocytes, eosinophils decreased significantly (p<0.05). Magnesium concentration, creatine phosphokinase, alanine transaminase, aspartate transaminase, phosphorus, red blood cells, hemoglobin, hematocrit, Mean cell volume, Mean cell hemoglobin did not show significant change (p>0.05), heart rate change, presence of arrhythmias and its type were significant (p<0.05). But, QRS pattern, T shape, P, QRS and T amplitude, S-T and Q-T interval waves had no significant change (p>0.05). No significant change was seen in control group. **CONCLUSIONS:** The results indicate that evaluating some biochemical, enzymatic, hematological and electrocardiography changes can be helpful in the diagnosis of hypocalcemia.

Introduction

In goats, a depression in serum levels of calcium and phosphorus occurs similar to that in cows but in ewes no such depression occurs at lambing and the intervention of a precipitating factor appears to be necessary to further reduce the serum calcium level below a critical point. Milking goats become affected mostly in the 4-6 years age group (Kenneth et al., 2011). Milk fever may occur in does and goats either prepar-

tum or postpartum because they have both the potential for high milk production and relatively large fetoplacental requirement associated with multiple births (Andreson et al., 2009). Normal parturition in goats is accompanied by mild hypocalcemia (Smith and Sherman 2011). The economic losses of milk fever include milk fever relapses, dystocia and reproductive diseases (Radostits et al., 2014). Mostaghni et al studied experimental hypocalcemia by EDTA on sheep in 2012, while Desmecht et al studied on calf in 1995, and Mellau et al induced the disease in cow and studied it in 2001. Since no research has been done about experimental hypocalcemia for goat by" EDTA infusion", the results of this study can reveal new information related to goat hypocalcemia as species of ruminant and beyond that in one of the Iranian original breeds. The origin of Markhoz goat is Kurdistan province in Iran. They are used as multipurpose animals for the production of milk, meat, hair and hide (Farshad et al., 2008). The aim of this study was to investigate the physiological effects of experimentally induced hypocalcemia on electrocardiography, hematology and serum biochemical changes in Markhoz breed goat.

Materials and Methods

10 female goats of Markhoz breed, weighing 25-30 kg, aged 1.5 to 2 years-old were selected.

Using history and ultrasonography their non-pregnancy was confirmed. Transabdominal ultrasonography was conducted using a real-time B-mode scanner (Emp-820 plus vet, Shenzhen Empror Electronic Technology Co. Ltd., China) equipped with 5 MHz convex transducer (Hesselink and

Taveme 1994, Raja et al., 2011, Karaddaev 2015). 10 goats were divided in two groups, the experimental group (n=5) and control group (n=5).

A 4.6% Na 2EDTA (Merck nr. 8418 Proanalysi, E. Merck) solution was prepared by dissolving 46 gr of salt in 1 liter of sterile distilled water and bringing its PH to 7.4 using sodium hydroxide. In order to induce hypocalcemia, a standardized flow rate of 1.2 ml/kg per hour of a 4.6% solution Na2EDTA was infused for 5 goats (experimental group) intravenously. Simultaneously with any EDTA solution infusion, the heart sounds, heart rate, rectal temperature, rumen activity and respiratory rate were controlled. Intravenous EDTA solution was stopped when the goats showed clinical signs of circulatory collapse manifested by cold extremities, increased heart rate, tachypnea, accumulation of mucus exudation in the nostrils, salivation, ruminal stasis, tympany, ataxia and sternal recumbency. Thereafter, goats were allowed to recover spontaneously from EDTA induced hypocalcemia. 5 healthy goats received 0.9% saline solution (IV) as control group in similar conditions. 10 ml blood sample of jugular vein was collected from two experimental and control groups before and after infusion, 8 ml of which was maintained in tube without anticoagulant for biochemical tests and the remaining 2 ml was maintained in evacuated heparine tubes for hematologic tests.

Electrocardiogram was recorded in standing position from two control and experimental groups before and after infusion, using electrocardiography device (model ECG2000 Bionet made in Korea) on base-apex lead. The paper speed was adjusted to 25 mm/ sec and calibration was 10 mm/ mV. For blood enzyme and biochemical

tests, first the blood samples were centrifuged to separate their serum, then serum parameters including calcium, phosphorus, magnesium, potassium, glucose, and ALT, AST, CPK and LDH enzymes were measured by Auto-chemistry Analyzer, (Model DIRUI, CS-T240 made in China) and potassium was measured with (Fater Electronic device, model 620 made in Iran) by flame Photometric method. Blood parameters including the white blood cells, the red blood cells, hemoglobin, hematocrit, MCV, MCH, MCHC and differential count of white blood cells were measured. To study electrocardiography changes, heart rate, the presence of arrhythmias and its type, QRS wave pattern, T wave shape, P amplitude, S-T interval, QRS waves amplitude, T wave amplitude and Q-T interval, were measured.

Initially data were entered in Microsoft Excel and then imported to GraphPad prism (GraphPad Software Inc., USA) version 3.0 where descriptive statistics (Mean, standard deviation of the mean) of the biochemical, hematologic and electrocardiographic analytical variables were determined. The difference between the mean of the variables before and after infusion was analyzed statistically by t- test and arrhythmia by using a nonparametric test of Wilcoxon, and p≤0.05 was considered significant.

Results

Results obtained from the study for two experimental and control groups has been shown separately in Tables 1 to 6 before and after infusion (after hypocalcemia induction) as the average standard deviation for any of the biochemical, hematologic, electrocardiographic parameters.

As shown in Table 1, decrease in calci-um concentration, LDH, potassium, and increase in glucose concentration before and after infusion was significant but magnesium concentration, CPK, phosphorus, ALT, AST has not shown significant change.

As shown in Table 3, the white blood cells, lymphocytes, eosinophils decreased significantly, but the red blood cells, hemoglobin, hematocrit, MCV, MCH, MCHC did not show significant change; as shown in Table 5, heart rate, presence of arrhythmias and its type also had significant change. But, QRS wave pattern, T wave shape, P, QRS, T wave amplitude, S-T and Q-T interval had no significance statistically.

In experimental group before infusion, arrhythmias were seen only in a case which was atrial fibrillation. But after induction of the hypocalcemia, the arrhythmias were diagnosed in all 5 goats and the diagnosed arrhythmias were atrial fibrillation in 3 cases, sinus arrhythmia as well as atrial fibrillation in 1 case and atrial premature beat with atrial fibrillation was seen in 1 case.

In control group before and after infusion no arrhythmias were diagnosed in 3 cases, but in 1 case before infusion, sinus arrhythmia and in another case, the second-degree atrioventricular block were diagnosed though no difference was observed compared to before infusion in 2 cases.

Regarding P value obtained from Wilcoxon test for arrhythmia in experimental group, it can be concluded that among arrhythmias, only the arrhythmia of atrial fibrillation with P=0.046 was less than 0.05 and is significant, it means that before and after infusion, it is different.

Discussion

EDTA solution with its chelating proper-

Table 1. The mean ± SD of biochemical parameters in test group. [a,b] Mean with different superscript on the same row are significantly different (p≤0.5).

Time	Ca (mg/dl)	Mg (mg/dl)	K (mg/dl)	P (mg/dl)	GLu (mg/dl)	AST (IU/L)	ALT (IU/L)	CPK (IU/L)	LDH (IU/L
Before	9.52±0.42a	2.54±0.56	4.92±0.38a	4.26±1	54±10	80 ±15	15 ±4.2	147±29	629±685a
After	6.16±1.5b	2.82±1.4	3.82±0.1b	4.7±1.4	61±12	77.8±14	15.2±3.7	192.6±63	608.8±65b

Table 2. The mean ± SD of biochemical parameters in control group.

Time	Ca (mg/dl)	Mg (mg/dl)	K (mg/dl)	P (mg/dl)	GLu (mg/dl)	AST (IU/L)	ALT (IU/L)	CPK (IU/L)	LDH (IU/L
Before	9.58±0.17	2.52±0.37	4.16±015	452±1.2	65±14	86±12	18.6±4	141.8±1	778.2±14
After	9.5±0.1	2.6±0.4	4.18±0.08	4.36±1.1	69±14	86±11	18.2±4.0	144.2±12	767±158

Table 3. The mean ± SD of hematologic parameters in test group. [a,b] Mean with different superscript on the same row are significantly different (p≤0.5).

Time	WBC (103/mm3)	RBC (106/mm3)	Hb (g/dl)	PCV (%)	MCV (Fl)	MCH (pg)	Lym. (103/mm3)	Neut. (103/mm3)	Eos. (103/mm3)
Before	19±1.53a	16.2±1.0	8.46±	25.3±4	15.5±1.9	5.17±o.6	11.2±1.9a	5 ±1.4	2.8±0.2a
After	16±2.5b	16±.82	8.3±1.5	24.9±4.	15.3±2.5	5.1±0.8	9.0±2.1b	5.3±1.0	2.2±0.2b

ties causes calcium in the blood to be away from the body's reactions, as a result hypocalcemia occurs. This property of EDTA was used in the study, and by injecting it, hypocalcemia conditions were induced.

In this survey, also calcium concentration was decreased from 9.52 mg/dl to 6.16 mg/dl (p<0.05) which confirmed the results obtained from a study in experimental hypocalcemia on sheep (Mostaghni et al., 2012), a study on calf (Desmecht et al., 1995), and one study's results on cow (Mellau et al., 2001).

Glucose concentration was changed from 54.6 mg/dl to 61 mg/dl after infusion (p<0.05) which was similar to results obtained from the other studies (Martinez et al., 2014 and Mostaghni et al., 2012).

Clinical signs observed by infusion EDTA and appearance of hypocalcemia in the study, including weakness, tremor and tetany causes the stress in experimental group, whereas this clinical signs were not observed in control group. The stress leads to increase in the secretion of adrenal corticotrophin hormone (ACTH) from the pituitary followed by increase in the secretion of cortisol from the cortex of adrenal gland.

Following the cortisol secretion increase, provocation for action of gluconeogenesis takes place in amount of 6 to 10 times, and thus promotes the formation of glucose. Cortisol also causes average decreases in the use of glucose by cells throughout the body (John et al., 2016). CPK enzyme was not significant after hypocalcemia induction (p>0.05), though the present result confirms Desmecht's et al., 2012 study on calf. Remarkable changes in the enzymes concentration are mostly related to skeletal muscles and cardiac muscle, and the damages to them. In the present study all goats of experimental group were not recumbent but tetany was observed in 5 goats in which CPK increase can be related to tetany caused by hypocalcemia. ALT and AST enzymes did not show significant change after inducing hypocalcemia (p>0.05), the results of which are similar to the those obtained in the study done by Mostaghni et al.,2012 and Desmecht et al., 1995 and the similarity was also observed about AST enzyme in

Table 4. The mean ± SD of hematologic parameters in control group.

Time	WBC (103/mm3)	RBC (106/mm3)	Hb (g/dl)	PCV (%)	MCV (Fl)	MCH (pg)	Lym. (103/mm3)	Neut. (103/mm3)	Eos. (103/mm3)
Before	18.9±4.4	16.3±0.5	8.9±0.3	26.8±0.9	16.4±1.0	5.4±0.3	11.2±1.9	5.4 ±3.4	2.3±0.9
After	18±4.0	15±0.4	8.9±0.5	26.9±1.5	16.8±1.1	5.6±0.3	11.0±1.7	4.7±2.9	2.5±0.7

Table 5. The mean ± SD of electrocardiographic parameters in test group. [a,b] Mean with different superscript on the same row are significantly different (p≤0.5).

Time	Heart rate/min.	ST duration (Sec)	QT duration (Sec)	QRS amplitude (mv)	T amplitude (mv)
Before	96.8±15.14a	0.176±0.036	0.22±0.02	0.36±0.11	0.24±0.21
After	111.8±12.49b	0.200±0.04	0.22±0.02	0.34±0.9	0.16±0.13

Table 6. The mean ± SD of electrocardiographic parameters in control group.

Time	Heart rate/min.	ST duration (Sec)	QT duration (Sec)	QRS amplitude (mv)	T amplitude (mv)
Before	100.4±23.86	0.176±0.022	0.19±0.033	0.34±0.11	0.34±0.11
After	100.2±25.44	0.168±0.018	0.19±0.033	0.40±0.10	0.36±0.13

Daniel's study (1979). Muscular enzymes including AST, CPK and LDH are entered in the blood stream with delay after muscular injuries incidence and for some time their amount remains high, for example, CPK amount reaches its maximum after 2 to 12 hours following muscular injury or AST amount remains high for more than a week after muscular injuries incidence. By continuing sampling in the coming hours, there was the possibility of obtaining conclusions other than the recent results that was not possible in the present study procedure to be examined. No significant change was seen for phosphorus concentration statistically (p>0.05) which confirms the results obtained by the other study (Daniel and Moodie, 1979). Change in the concentration of phosphorus plasma is subject to Parathoromone changes, so it can be concluded that the cause of various reports about being significant or not for phosphorus decrease simultaneously with calcium decrease, is due to the difference in reaction of parathyroid glands in animals tested. Also, the amount of hypocalcemic and phosphorus absorption and excretion by animals is regarded as important factors in blood phosphor changes,

so that phosphorus decrease was observed in some samples of the study, but the mean had no significance statistically.

LDH enzyme was decreased after infusion and the change was significant statistically (p<0.05) which does not confirm the results obtained by the studies done by Desmecht et al., 1995 indicating non-significance of the enzyme changes.

This enzyme exists in all tissues in large quantities, some anticoagulant substances such as EDTA and Oxalates inhibit the enzyme activity indirectly and decrease its amount.

In this study LDH amount had significant decrease unexpectedly, this decrease was not observed in control group, and the reason for this is probably experimental hypocalcemia by injecting EDTA solution that causes the LDH to be decreased indirectly. Therefore, using EDTA solution to measure LDH enzyme changes in experimental hypocalcemia is not appropriate and other methods can be applied.

Potassium was decreased after hypocalcemia induction (p<0.05) that is similar to another study (Martinez et al., 2014). Calcium decrease was followed by parathyroid

hormone which reduces reabsorption of potassium in proximal tubular and its quick excreting in the urine.

No significant statistical change was observed in Magnesium after hypocalcemia induction (p>0.05), which confirms the results obtained in a similar study (Mellau et al., 2001), but does not confirm the result of Martinez et al., (2014) and Yamagishi et al., (1999) in hypocalcemia induced with hemodialysis regarding increase in Magnesium.

Magnesium slightly increased in the study which can be related to parathyroid hormone that increases reabsorption of Magnesium ions and hydrogen ions.

White blood cells, the number of lymphocytes and eosinophils of the blood decreased after hypocalcemia induction and the decrease was significant statistically (p<0.05). Cortisol decreases the number of lymphocytes, and subsequent eosinophils in the blood. The reason for this is that T lymphocytes produce eosinopoietine and eosinophil chemotactic anaphylactic factor (ECAF).The first protein to increase production of eosinophil in bone marrow and second protein is chemotaxic for eosinophil. This effect is mediated through the anterior pituitary and adrenocorticol hormones which are increased during stressful situation (Karpoor et al., 2011). Antihistamine and lyses' property of T lymphocyte, margination lymphocytes and eosinophils in blood vessels and establishing them in the tissues is caused by cortisol secretion, these effects start within a few minutes after cortisol secretion and are revealed within a few hours. Decrease in the number of lymphocytes, eosinophils causes the decrease in the number of white blood cells.

Other hematological parameters including the red blood cells, hemoglobin, hematocrit, MCV, MCH and neutrophil have not shown significant changes statistically (p>0.05); regarding the number of neutrophils and the red blood cells, it confirms the other study (Matinez et al., 2014).

Although the increase in the number of neutrophils was not significant statistically (p>0.05), decreasing neutrophil adhesion to the arteries inner walls and decreasing their migration from the reservoir circulating to the tissue caused by cortisol secretion resulted from stress can be the reasons for this increase (Kenneth et al., 2011).

Regarding the role of calcium on cardiac mechanical activity, it is of particular importance in electrocardiogram changes in which electrocardiogram parameter was evaluated before and after hypocalcemia induction in this study.

In the study, QRS wave pattern, T wave shape and P amplitude, in two groups did not show significant change before and after hypocalcemia induction. Heart rate changes after hypocalcemia induction had significant increase (p>0.05). Contractive strength of the heart muscle depends on Calcium ions concentration in extracellular fluids and decrease of extracellular calcium induces disorders for heart natural performances including decrease in contractive strength of the heart, tonicity decrease of the arteries wall leads to peripheral vascular dilation, decreases in blood pressure, increases in heart rate to compensate blood pressure decrease and decrease in intensity of heart sounds.

Moreover, in clinical examination during testing on animals, the heart sound intensity was decreased remarkably that confirms the studies in induced hypocalcemia with hemodialysis (Yamagishi et al., 1999).

T waves amplitude was decreased after

hypocalcemia induction but this decrease had no significant difference statistically (p>0.05). This decrease may be due to individual differences among animals. Changes in QRS waves amplitude had no significant difference before and after hypocalcemia induction (p>0.05). QRS complex indicates ventricles activity in which amplitude and shape depend on the animal heart status. It can be concluded that experimental hypocalcemia has no effect on QRS wave amplitude.

Q-T interval wave did not show significant change statistically before and after hypocalcemia induction (p>0.05), that has no similarity with the results obtained in the study which indicate significant increase in Q-T interval in hypocalcemia (Daniel and Moodie 1979).

Q-T interval wave was increased after hypocalcemia induction, but this increase was not significant statistically (p>0.05).

S-T segment is the distance between ventricular depolarization and ventricular repolarization,this distance is extended in electrolyte abnormalities. Distance extension of S-T segment in experimental hypocalcemia can be a helpful diagnostic tool (Radostits et al., 2014).

Although no significant change was observed in this study, surveying electrocardiography with delay after hypocalcemia may show this change.

Q-T interval has considerable importance in condition of electrolyte changes, so that it increases in hypocalcemia and hypokalemia (Radostits et al., 2014). In this study, Q-T interval increase in one of the goats in experimental group was significant so that the goat's calcium concentration was less than all goats, therefore, it can be concluded that in small amounts of calcium, Q-T interval

can be increased.

In this study, there was arrhythmia in one goat in experimental group before hypocalcemia (Atrial Fibrillation). But after inducing hypocalcemia, arrhythmia appeared in all goats. Atrial fibrillation arrhythmia was diagnosed in 3 cases, and atrial fibrillation with sinus arrhythmia in one case, as well as atrial fibrillation with premature atrial beat in another case that confirms the results obtained by Dehkordi et al., (2014) in studying arrhythmia on high and low-yielding dairy cows, in which blood calcium decrease was introduced as an agent causing arrhythmia. Atrial fibrillation was the main arrhythmia type observed after hypocalcemia induction which was significant statistically. Appearance of this arrhythmia type is mostly related to disorder in heart performances and anatomical damage plays no role in its incidence and it mostly associates with metabolic disease and electrolyte abnormalities.

As calcium has a significant role both in the conduction heart system and in heart muscle performance, it can be deduced that calcium amount decrease may lead to appearance of this type of arrhythmia (Bardley and Cunningham 2012). Findings obtained from biochemical, hematologic, electrocardiographic changes caused by experimental hypocalcemia of goat can provide a more complete understanding for pathophysiologic and metabolic process for this disease.

Acknowledgements

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بررسی تغییرات پارامترهای الکتروکاردیوگرافی، هماتولوژی و بیوشیمیایی در هیپوکلسمی تجربی در بزهای نژاد مرخز

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چکیده

زمینه مطالعه: هیپوکلسمی یکی از مهمترین بیماری‌های متابولیک در گاو، گوسفند و بز پیرامون زایمان است و به علت کاهش عیار سرمی کلسیم خون اتفاق می‌افتد. **هدف:** هدف از انجام این مطالعه بررسی تغییرات بیوشیمیایی، هماتولوژی و الکتروکاردیوگرافی در شرایط هیپوکلسمی تجربی در بزهای نژاد مرخزمی‌باشد. **روش کار:** دراین مطالعه ۱۰ بز نژاد مرخز به دو گروه تست و کنترل تقسیم شدند. (هرگروه ۵ بز) در گروه آزمایش باتزریق محلول اتیلن دی آمین تترا استات سدیم ۴/۶٪ بصورت وریدی علائم بالینی هیپوکلسمی با تغییر در ضربان قلب و زمین گیری ایجاد شد. در گروه کنترل نیز درشرایط مشابه سرم فیزیولوژی تزریق شد. در هر دو گروه قبل و پس از تزریق نوارالکتروکاردیوگرام در اشتقاق قاعده‌ای راسی و نمونه خون از ورید وداج اخذ شد. الکتروکاردیوگرافی و پارامترهای کلسیم، پتاسیم، منیزیم، فسفر، گلوکز، آنزیم‌های CPK, AST, ALT درسرم و پارامترهای هماتولوژیک شامل RBC, WBC Diff leukocyte, PCV, Hb, MCV, MCH, MCHC در خون اندازه گیری شـدند. **نتایج:** نتایج این مطالعه در گروه آزمایش کاهش معنی‌داری در عیار کلسیم، LDH، پتاسیم، و افزایش معنی‌داری در غلظت گلوکز نشان دادند. تعداد گلبول‌های سفید، تعداد لنفوسیت و تعداد ائوزینوفیل خون کاهش معنی‌داری رانشان دادند. و مقادیر منیزیم، CPK، فسفر، AST،ALT، تعداد گلبول‌های قرمز، مقدار هموگلوبین، میزان هماتوکریت، MCH،MCV و MCHC تغییر معنی‌دار را نشان ندادند. تعداد ضربان قلب و وجود آریتمی و نوع آن نیز دارای تغییرات معنی‌دار بود. اما تغییرات الگوی امواج QRS، شـکل موج T، ارتفاع امواج P، QRS و T فاصله امواج S–T و Q–T از لحاظ آماری معنی‌دار نبودند. در گروه کنترل هیچ تغییر معنی‌داری مشاهده نشد. **نتیجه‌گیری‌نهایی:** نتایج حاصل از این مطالعه نشان می‌دهند که در تشخیص بیماران مبتلا به هیپوکلسمی، ارزیابی برخی از تغییرات بیوشیمیایی، آنزیمی، هماتولوژی و الکتروکاردیوگرافی می‌توانند کمک کننده باشند.

واژه های کلیدی: هیپوکلسمی تجربی، الکتروکاردیوگرافی،هماتولوژی، بیوشیمیایی، بز مرغز

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