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Diagnosis of Hypomagnesaemia in Heifer calves and Experimental Treatment with Magnesium Oxide and Basil &Thyme

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Abstract

Hypomagnesaemia was reported as a common clinical problem in calves in Egypt. The present study was conducted to diagnose hypomagnesemia in calves and evaluate the treatment of diseased cases by using Mg oxide, Basil &Thyme as food additives. Currently, a total of 1000 female Holstein dairy calves imported from Europe to the farm of the Faculty of Agriculture- Sakha- Agriculture of Research Institute aged 7-9 months, out of these calves 450 have nervous manifestations suspected hypomagnesemia and confirmed by detection of serum magnesium. After symptomatic treatment of diseased cases, calves were separated and divided into 4 groups each one containing 50 calves; the apparently healthy group (control) fed on a balanced ration according to NRC, diseased calves fed on a ration with a high protein diet, treated calves fed on a balanced ration with the addition of Mg oxide 10gm/animal/day for 2 months and treated calves fed on balanced ration with the addition of Basil and Thyme 2% (2 kg/100 kg ration) for 2 months**.** According to the findings, there was a significant decrease in sodium, phosphorus, magnesium, ionized magnesium, calcium, ionized calcium, GSH, SOD, TAC, glucose, insulin, cortisol, and PTH levels while, there was an increase in potassium, liver, and kidney enzymes, CPK, T_4, in diseased group comparing with the control one. Treating diseased animals with magnesium oxide or Basil and Thyme revealed improvement of minerals, antioxidants, glucose, insulin, cortisol, PTH levels, and a decrease in liver & kidney enzymes, T4, MDA, & CPK levels within normal range.

Keywords: ionized magnesium, ionized calcium, CPK, protein, parathyroid

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Introduction

Mineral deficiencies may be brought on by poor feed quality, decreased mineral absorption or assimilation in the body, or increased mineral demand during rapid growth. Mineral-deficient feed and diets hinder young animals' growth and suppress their appetite, immunity, and nutrition absorption. They also make them more susceptible to infectious diseases (Radwińska and Żarczyńska, 2014, Galbat et al., 2021). According to (Constable et al., 2017), hypomagnesemia is a significant metabolic disorder that primarily affects calves and cattle. It is caused by a primary dietary deficiency in magnesium, a crucial component of over 300 enzymes regulating numerous bodily processes. These include ATPases, kinases, and phosphatases; RNA, DNA, and protein synthesis; acting as modulators of synaptic transmission in skeletal muscle; and a regulator of membranes (Urdaz et al., 2003). The disorder is characterized by nervous signs such as convulsions, initial excitation, bellowing, muscle spasms, tetany, and rapid death may happen to the animal at the end. It corresponds to low levels of magnesium in the blood and cerebrospinal fluid. According to (Martens & Schweigel, 2000), hypomagnesemia has a rapid clinical course and a high mortality rate, its development depends on a balance between Mg intake and loss, which is influenced by both host environmental and host factors, excessive nitrogen and feeding animals on high protein diets which interfere with absorption of magnesium in the rumen. Due to the lack of a readily accessible magnesium reserve, calves are extremely sensitive to changes in the input-output balance of magnesium in their bodies. also, ionized magnesium is the biologically active form of Mg in the cells so it's more accurate in detecting Mg deficiency in serum than total Mg (Lopez et al, 2006).

Food additives are a fantastic way to increase the efficiency of feeding and animal production (Abdou, 2001). Research on animals is therefore seeking alternatives for these chemicals. Essential oils are one type of plant extract that can be utilized as a natural feed supplement in ruminant nutrition (Yang et al., 2010). In addition, (Magi and Sahk, 2003) noted that herbal medicine is currently a developing field of alternative medicine. Many of the active ingredients included in manufactured medicinal products are derived from plant mixtures and have a variety of uses. It is well known that plants are more natural, secure, and non-poisonous than chemical arrangements (Marwan and Mousa 2021).

According to, (Ben-Jabeur et al, 2015) the thyme plant and oil are among the top 10 most commercialized oils in the world and are widely used as natural food preservatives due to their intense antibacterial, antioxidant, and antifungal properties. Thyme is also used in medicine as antiviral, antispasmodic, tonic, cramp, spasm, nervousness, diaphoretic, diuretic, and cancer treatment (Duck et al, 2002).

Basil is the common name for the culinary herb Ocimum basilicum of the family Lamiaceae (Labiatae) which is one of the largest families with over 5,000 aromatic and medicinal plant species and essential oils that may be extracted for a variety of uses (Gurib-Fakim, 2006).

In ruminants, using Mg supplements involves organic and inorganic elements, and in this regard, an ideal Mg supplement should be palatable, cheap, and easily absorbed from the rumen (Odette, 2005; Schonewille, 2013) For this reason Magnesium oxide (MgO) is one of the most used magnesium supplements (Schonewille et al., 2008); Maintaining magnesium in the normal physiological ranges (Mellado et al., 2004).

In the present study, the imported calves were suffering from magnesium deficiency. Basil & thyme are used widely for the treatment of spasms, convulsions, and nervous manifestation (Duck et al, 2002). Also due to their high magnesium content according to AOAC, we decided to use them in the treatment of hypomagnesemia in comparison to the traditional Mg salts used in the treatment of hypomagnesemia (MgO).

Due to the high economic importance of calves this study aimed to

Clinical and laboratory diagnosis of hypomagnesemic calves and estimating the levels of some minerals (Magnesium, ionized magnesium, calcium, ionized calcium, phosphorus, sodium, potassium), some oxidants, and antioxidants parameters, some hormones, glucose, liver, and kidney enzymes.

Expermintal treatment of hypomagnesemic calves by using traditional treatment Mg oxid and detecting its effect on some minerals, oxidants, antioxidants parameters, hormones, glucose, liver, and kidney enzymes.

Expermintal treatment of hypomagnesemic calves by adding Basil and Thyme on the ration as food additives and detecting its effect on some minerals, oxidants and antioxidant parameters, some hormones, glucose, liver, and kidney enzymes.

Material and Methods

This work was carried out at the farm of the Faculty of Agriculture- Sakha-Agriculture of Research Institute. A total of 1000 female Holstein calves were imported from Europe ranging from 7-9 months, and weighing 110–120 kg, the exported calves were fed on a ration with a high protein diet. out of them, 450 calves suffered from nervous manifestations including muscular tremors, frothing at the mouth, frequent urination, and defecation. The signs suspected hypomagnesemia which confirmed laboratory. hypomagnesemic calves were symptomatically treated then 150 separate calves were used for the study. The work was carried out during the period from March 2021 to May 2021. Calves were divided into three groups compared to a control group without any clinical signs. Each group consists of 50 calves; control group calves fed on a balanced ration according to NRC, diseased calves fed on a high protein diet, treated calves fed on a balanced ration according to NRC with the addition of Mg oxide 10gm/animal/day for 2 months, treated calves fed on a balanced ration according to NRC with the addition of Basil and Thyme 2% (2 kg/100 kg ration) for 2 months.

This work was done according to the ethics of the Faculty of Veterinary Medicine, South Valley University with approval number “VM/SVU/23(2)-05”.

Samples

Four types of samples were collected for this study: blood, ration, water, Basil & Thyme samples. The blood samples (10 ml) were collected from the jugular vein of each calve in clean glass vials and converted to a serum that was stored at -20 ̊c until testing.

The separated sera are used for the detection of some minerals (Mg, Ca, ph (mg/dl), ionized Mg& Ca, Na, and K (Mmol/l) (A.O.A.C, 2015), oxidants MDA (µmol/ml) (Ohkawa et al., 1979), antioxidants included TAC (u/ml) (Koracevic et al., 2001), SOD (u/ml) (Nishikimi et al., 1972), and GSH (u/ml) (Paglia and Valentine, 1967), liver and kidney enzymes, glucose (Young, 2001) by using a (Spectro uv-vis double beam pc scanning spectrophotometer uvd-2950) and using of ion selective electrode sensa core` st-200 aqua electrolyte analyzer.

Insulin and cortisol levels were determined by the ELISA method, according to A.O.A.C. (2015), T_3, T_4, and PTH according to (Thakur et al., 1997).

Samples collected from food, water (fresh and clean drinking water was supplied ad-libitum), and Basil & Thyme delivered to the studied cases during the experiment were used to analyze its contents.

The prepared ration delivered to calves consisted of green clover 20%, dress berseem 80% yellow corn 18%, roughages 23%, vitamins 1%, salt 1%, bran 30%, barely 25% and limestone 2%, and analysis of samples of the ration, water and Basil and Thyme delivered to animals were done in Chemistry and Nutritional Deficiency Department, Animal Health Research Institute, Giza and showed in Tables 1, 2 &3.

Table 1: Ration analysis

Items	Examined animal ration
Total protein	14.5%
Total fat	2%
Humidity	12%
Ash	10%
Fiber	17%
Carbohydrate	44.5%
Total energy-calories	2584 kcal/kg ration
Ca	0.7mg%
Ph	0.33 mg%
Mg	0.22mg%

Table 2: Water analysis

Items	Examined group
Appearance color	Clear
PH value	7.3
Conductivity (ms/m)	460
Calcium (mg/l)	50
Calcium carbonate (mg/l)	125
Magnesium mg/l	6.0
Magnesium carbonate	21
TSS (total suspended solids)	60
TDS (total dissolved solids)	470
TS (total solids)	530
Chloride (mg/l)	100
Nitrate & nitrite (mg/l)	-ve
Sulphate (mg/l)	77
Phosphorus (mg/l)	-ve
Phosphate (mg/l)	-ve
Free chlorine (mg/l)	-ve
Cadmium	-ve
Lead	-ve

Table 3: Thyme analysis

Items	%
Protein	7.0
Fat	1.7
Humidity	8.0
Ash	81

Fiber	**14**
Carbohydrates	**28.3**
Sugar	**24**
Salts (mg/100 mg)	
Na	**4.0**
K	**609**
Fe	**17.4**
Ca	**405**
Ph	**106**
Mg	**160**
Zn	**1.8**
Cu	**0.46**
Mn	**1.6**
Amino acids (g/100mg)	
Tryptophan	**0.112**
Threonine	**.18**
Lucien	**.28**
Licin	**.11**
Methionine	**0**
phenyl alanine	**0**
Vitamins	**Iu**
A	**4700**
D	**0**
E	**0**
K	**0**
C	**185mg/dl**
B	**0**

Table 4: Basil analysis

Items	%
Protein	**3.2**
Fat	**0.6**
Humidity	**51.95**
Ash	**40**
Fiber	**1.6**
Carbohydrates	**26.5**
Sugar	**2.4**
Salts (mg/100 mg)	
Na	**4.0**
K	**295**
Fe	**3.17**
Ca	**177**
Ph	**56**
Mg	**640**
Zn	**0.3**
Cu	**0.38**
Mn	**1.15**
Amino acids (g/100mg)	
Tryptophan	**0.48**
Threonine	**.2**
Lucien	**.18**
Licin	**.12**
Methionine	**0.17**
phenyl alanine	**0.2**
Vitamins	**Iu**
A	**5275**
D	**0**
E	**0**
K	**0**
C	**10mg/dl**

B_{12} **B_6**	**0.03** **0.18**

Assessment of therapeutic response

The therapeutic response of diseased animals was assessed after a five-day treatment with SC injection of 2 ml/kg BW 20% magnesium sulfate (El-Nasr Company for chemicals, Egypt) combined with IV injection of 100 ml calcium magnesium boro gluconate (Canimag, Pharmaswide, Egypt). In addition, 10 ml of the Vitamin B complex (antoplex, Nile Company, Egypt) was given IM for 5 days and 2 gm Mg sulfate was given orally for 5 days (Naik et al., 2010). A single IM injection of 0.1mg/kg BW xylazine (Xyla-Ject®, Adwia Pharmaceuticals, Egypt) was given for tetanic cases.

Statistical analysis

The obtained data were statistically analyzed after the methods described by (Snedecor and Cochran, 1980) by using one-way ANOVA and statistical package for social science (SPSS) computer program, and Duncan's multiple range test was used to compare the means (Steel and Torrie, 1980).

Results

Clinical findings

Clinical examination of diseased animals revealed nervous manifestations including hyper-irritability, tetany, opisthotonos, and stiffness resulting in severe convulsions.

Biochemical findings

Biochemical Changes of minerals in hypomagnesemic calves, treated calves with MgO, and basil & thyme.

There was a significant decrease in Na, Ca, ionized Ca, Mg, ionized Mg, and Ph levels in the diseased group which was(122 mmol/l, 8 mg/dl, 0.72 mmol/l, 1.3 mg/dl, 0.3 mmol/l, 4.3 mg/dl) when compared to control groups which were (137 mmol/l, 10.4 mg/dl, 1.05 mmol/l, 3mg/dl, 0.51 mmol/l, 6.3 mg/dl); respectively while there was a significant increase in K level in the diseased group which was (7 mmol/l) when compared to control one which was (4 mmol/l).

There was a significant increase in Na, Ca, ionized Ca, Mg, ionized Mg, and Ph levels in the treated group with Mg oxide which was (133 mmol/l, 9.2 mg/dl, 1.15 mmo/l, 2.9 mg/dl,0.6 mmol/l, 5.6 mg/dl), and Basil & Thyme which were (135 mmol/l, 10 mg/dl, 1.2 mmol/l, 2.5 mg/dl, 0.6 mmol/l, 6 mg/dl); respectively when compared to the diseased group which was (122 mmol/l, 8 mg/dl, 0.72 mmol/l, 1.3 mg/dl, 0.3 mmol/l, 4.3 mg/dl) while there was a significant decrease in K level in treated groups which were (4.4 & 4 mmol/l) when compared to diseased which was (7 mmol/l) at the level of p value ≤ 0.05 as shown in Table 5.

Table 5: Biochemical Changes of minerals in hypomagnesemic calves, treated calves with MgO, and basil & thyme.

	Control	Diseased	Treated w Mg	Treated w basil& thyme
Na(mmol/l)	**137± 1.4**	**122±1.6[a]**	**133±2.4[b]**	**135±1.8[c]**
K (mmo/l)	**4± 0.1**	**7± 0.2[a]**	**4.4± 0.1[b]**	**4± 0.1[c]**
Ph (mg/dl)	**6.3± 0.1**	**4.3± 0.1[a]**	**5.6± 0.2[b]**	**6± 0.1[c]**
Ionized Ca(mmol/l)	**1.05± 0.1**	**0.72± 0.01[a]**	**1.15± 0.01[b]**	**1.2± 0.11[c]**

Ca (mg/dl)	**10.4± 0.1**	**8± 0.1[a]**	**9.2± 0.1[b]**	**10± 0.1[c]**
Ionized Mg(mmol/l)	**0.51±0.02**	**0.3±0.03[a]**	**0.6± 0.02[b]**	**0.6± 0.02[c]**
Mg (mg/dl)	**3± 0.03m**	**1.3± 0.1[a]**	**2.9± 0.1[b]**	**2.5± 0.04[c]**

Data presented as mean ± SE
-the letters a, b & c are used to denote the significant difference as follows
a for the significant difference between the diseased group with control one
b for the significant difference between Mg oxide-treated group with diseased group
c for the significant difference between Thyme & Basil treated group with diseased group

Liver enzymes change in hypomagnesemic calves, treated calves with MgO, basil & thyme.

There was a significant increase in the level of ALT, AST, and ALP in the diseased group which was (78, 117& 231 u/l) when compared to the control one which was (40, 73& 113 u/l); respectively and there was a significant decrease in their level in treated groups with Mg oxide and Basil& Thyme groups which were (53, 91& 134 and 43, 71& 126 u/l); respectively when compared to diseased ones at the level of p value ≤ 0.05 as shown in Table 6.

Table 6: Liver enzymes change in hypomagnesemic calves, treated calves with MgO, basil & thyme.

Groups Items	Control	Diseased	Treated w Mg	Treated w basil& thyme
ALT (u/l)	**40±1.1**	**78±1.5[a]**	**53±1.3[b]**	**43±1[c]**
AST (u/l)	**73± 2.5**	**117± 2[a]**	**91± 1.8[b]**	**71± 2.2[c]**
ALP (u/l)	**113± 4.2**	**231± 1.3[a]**	**134± 2.5[b]**	**126± 2.8[c]**

-Data presented as mean ± SE
-the letters a, b & c are used to denote the significant difference as follows
a for significant difference between the diseased group and the control group
b for significant difference between Mg oxide-treated group and the diseased group
c for a significant difference between Thyme & Basil treated group and the diseased group

Changes in kidney enzymes and CPK in hypomagnesemic calves, treated calves with MgO, basil & thyme

There was a significant increase in the level of urea, creatinine, uric acid, and CPK in the diseased group which was (63, 2, 7 mg/dl& 46.4 u/l) when compared to the control group which was (33, 1.2, 5 mg/dl& 15.4 u/l); respectively and there was a significant decrease in their level in treated groups with Mg oxide and Basil & Thyme which were (45, 1.3, 6.2 mg/dl& 21.5u/l and 38, 1.2, 5 mg/dl, 27.1u/l); respectively when compared to diseased ones which were (63, 2, 7 mg/dl& 46.4 u/l) at the level of p value ≤ 0.05 as shown in Table 7.

Table 7: Changes in kidney enzymes in hypomagnesemic calves, treated calves with MgO, basil & thyme.

Groups Items	Control	Diseased	Treated w Mg	Treated w basil& thyme
Urea(mg/dl)	**33± 0.9**	**63±1.6[a]**	**45±1.6[b]**	**38±1.6[c]**
Creatinine (mg/dl)	**1.2± 0.04**	**2± 0.4[a]**	**1.3± 0.03[b]**	**1.2± 0.03[c]**

Uric acid (mg/dl)	5± 0.07	7± 0.2[a]	6.2± 0.1[b]	5± 0.1[c]
CPK (u/l)	15.4± 1.02	46.4± 2[a]	21.5± 0.8[b]	27.1± 2.8[c]

Data presented as mean ± SE
-the letters a, b & c are used to denote the significant difference as follows
a for significant difference between the diseased group and the control one.
b for significant difference between the Mg oxide-treated group and the diseased group.
c for significant difference between Thyme & Basil treated and diseased groups.

Changes in oxidants and antioxidant levels in hypomagnesemic calves, treated calves with MgO, basil & thyme.

There was a significant decrease in the level of GSH, SOD & TAC in the diseased group which was (19.7mu/ml, 2.4u/ml& 220 u/ml) when compared to the control group which was (45.5mu/ml, 6.5u/ml& 595 u/ml); respectively while there was a significant increase in MDA which was (21 µmol/ ml) in the diseased group when compared to control one (9.2 µmol/ ml).

There was a significant increase in the levels of GSH, SOD & TAC in treated groups with Mg oxide and Basil & Thyme which were (28mu/ml, 3.8u/ml& 303u/ml, and (42mu/ml, 6.6u/ml& 590 u/ml); respectively when compared to the diseased group which was (19.7mu/ml, 2.4u/ml& 220 u/ml) while there was a significant decrease in MDA in Basil and Thyme treated group which was (9.4mmol/l) and non-significant decrease in Mg treated group which was (19µmol/ ml) when compared to diseased one which was (21 µmol/ ml) at the level of p value ≤ 0.05 as shown in Table 8.

Table 8: Changes in oxidants and antioxidants in hypomagnesemic calves, treated calves with MgO, basil & thyme.

	Control	Diseased	Treated w Mg	Treated w basil& thyme
GSH (mu/ml)	45.5± 1.9	19.7±0.85[a]	28± 0.72[b]	42± 1.9[c]
SOD(u/ml)	6.5± 0.3	2.4± 0.2[a]	3.8± 0.2[b]	6.6± 0.2[c]
TAC(u/ml)	595± 5	220± 4.5[a]	303± 6.5[b]	590± 8.6[c]
MDA (µmol/ ml)	9.2± 0.4	21± 0.9[a]	19± 0.5	9.4± 1.2[c]

Data presented as mean ± SE
-the letters a, b & c are used to denote the significant difference as follows:
a for significant difference between the diseased group and the control one
b for significant difference between Mg oxide-treated group and the diseased group
c for significant difference of Basil & Thyme treated groups with diseased group

Changes in glucose, insulin, and cortisol levels in hypomagnesemic calves, treated calves with MgO, basil & thyme.

There was a significant decrease in the glucose, insulin, and cortisol levels in the diseased group which was (50mg/dl, 18Iu/ml& 10nmol/l) when compared to the control ones which was (72mg/dl, 26Iu/ml& 15nmol/l); respectively and there was a significant increase in treated group with Mg oxide and Basil& Thyme which were (68mg/dl, 22Iu/ml& 13.5nmol/l and 54mg/dl, 26 Iu/ml& 16nmol/l) when compared to diseased one which was (50mg/dl, 18Iu/ml& 10nmol/l) at the level of p value ≤ 0.05 as shown in Table 9.

Table 9: Glucose, insulin, and cortisol levels in hypomagnesemic calves, treated calves with MgO, basil & thyme.

	Control	Diseased	Treated w Mg	Treated w basil& thyme
Glucose(mg/dl)	72± 2	50± 2[a]	68± 1[b]	54± 1.7[c]
Insulin (Iu/ml)	26± 0.7	18± 0.4[a]	22± 0.7[b]	26± 0.3[c]
Cortisol (nmol/l)	15± 0.7	10± 0.3[a]	13.5± 0.2[b]	16± 0.4[c]

Data presented as mean ± SE
-the letters a, b & c are used to denote the significant difference as follows:
a for significant difference between the diseased group and the control one
b for significant difference between the Mg oxide-treated group and the diseased group
c for significant difference between thyme& basil treated group with diseased group

Hormonal changes in hypomagnesemic calves, treated calves with MgO, basil & thyme.

There was a non-significant change in T3 level between different groups while there was a significant increase in T_4 level which was (2ng/dl) in the diseased group when compared to the control one which was (1.5ng/dl) and there was a significant decrease in treated groups with Mg oxide and Basil& Thyme which were (1.6& 1.4ng/dl); respectively when compared to diseased one which was (2ng/dl).

There was a significant decrease in PTH level in the diseased group which was (5.6ng/dl) when compared to the control one which was (9.2ng/dl) and there was a significant increase in treated groups with Mg oxide and Basil& Thyme which were (9& 9.2 ng/dl) when compared to diseased one which was (5.6ng/dl) at the level of p value ≤ 0.05 as shown in Table 10.

Table 10: Hormonal changes in hypomagnesemic calves, treated calves with MgO, basil & thyme

	Control	Diseased	Treated w Mg	Treated w basil& thyme
T3(ng/dl)	52± 2	54± 2	52± 1.3	49± 0.9
T4 (ng/dl)	1.5± 0.1	2± 0.1[a]	1.6± 0.04[b]	1.4± 0.04[c]
PTH (ng/dl)	9.2± 0.2	5.6± 1.4[a]	9± 0.1[b]	9.2± 0.1[c]

Data presented as mean ± SE
-the letters a, b & c are used to denote the significant difference as follows:
a for significant difference between the diseased group and the control one
b for significant difference between Mg oxide-treated group and the diseased group
c for a significant difference in thyme & basil treated group with diseased group

Evaluation of treatment of hypomagnesemic calves with Mg salt and Basil and Thyme

The result of the obtained study revealed that there was only a significant difference in glucose, ALT and AST levels in Basil and Thyme treated group of calves with hypomagnesemia when compared to those treated with Mg salt while there was a significant difference in cortisol between groups for Mg treated one at the level of p ≤ 0.05 as shown in Table 11.

Table 11: Evaluation of treatment of hypomagnesemic calves with MgO, basil & thyme treatment.

	Mg salt	Basil and Thyme
Mg	2.9± 0.1	2.5± 0.04
Ca	9.2± 0.1	10± 0.1
P	5.6± 0.2	6± 0.1
K	4.4± 0.1	4± 0.1
Na	133±2.4	135±1.8
Ionized Mg	0.6± 0.02	0.6± 0.02
Ionized Ca	1.15± 0.01	1.2± 0.11
ALT	53±1.3	43±1[*]
AST	91± 1.8	71± 2.2[*]

ALP	**134± 2.5**	**126± 2.8**
Urea **Creatinine** **Uric acids** **CPK**	**45±1.6** **1.3± 0.03** **6.2± 0.1** **21.5± 0.8**	**38±1.6** **1.2± 0.03** **5± 0.1** **27± 2.8**
GSH **SOD** **TAC** **MDA**	**28± 0.72** **3.8± 0.2** **303± 6.5** **19± 0.5**	**42± 1.9** **6.6± 0.2** **590± 8.6** **9.4± 1.2**
Glucose **Insulin** **Cortisol**	**68± 1** **22± 0.7** **13.5± 0.2****	**54± 1.7*** **26± 0.3** **16± 0.4**
T_3 **T_4** **PTH**	**52± 1.3** **1.6±0.4** **9± 0.1**	**49± 0.9** **1.4±0.4** **9.2± 0.1**

*for significant difference with treatment with Mg salt
**for significant difference between Basil and Thyme

Discussion

Nutrients are very important for the general body's health. They provide the different tissues with the necessary raw ingredients. As cofactors for numerous enzymes, macronutrients have a particularly significant function in several metabolic processes (Nwosu, 2019). Vitamins, minerals, and other dietary sources of micronutrients are crucial micronutrients that are required at minute levels to stimulate various biological activities for cell metabolism in animals (Lee et al., 2002). For the survival of reproductive and productive animals, the composition and balance of minerals are important (Vázquez-Armijo et al., 2011). According to (Nwosu, 2019); minerals play a significant physiological and biological role. Decreased mineral feed levels, absorption, and bioavailability have undesirable impacts and health issues (Arshad et al., 2021).

Clinical signs of hypomagnesemia

The hyperirritability of the neurological system and the onset of tetany, which results in stiffness, opisthotonos, and severe convulsions were the clinical findings seen in the hypomagnesaemic calves in this investigation. The clinical symptoms that were seen were the same as those stated by (Cronin, 2006; Naik et al., 2010, Constable et al., 2017). When blood magnesium levels are substantially low, neurological symptoms tend to be reflections of cerebrospinal fluid magnesium concertation that are related to serum in a linear pattern (Martens and Schweigel 2000). This may also be explained by the fact that magnesium has a central nervous system influence by activating choline esterase, which breaks down acetylcholine; therefore, the central nervous system was hyper-irritable when magnesium levels were low. The age of most affected calves was also between 4 and 9 months; this may be explained by the fact that by five months of age, an animal's ability to absorb magnesium had declined to roughly 20% (Smith, 1996).

Another factor is that, unlike the metabolism of many other critical minerals, the homeostasis of magnesium is not regulated by a distinct hormonal system (Urdaz et al., 2003).

Biochemical changes due to hypomagnesemia in calves

Changes in minerals concentration due to hypomagnesemia

The result of the obtained study revealed that there was a significant decrease in Na, Ca, ionized Ca, Mg, ionized

Mg, and Ph levels in the diseased group when compared to control groups while, there was a significant increase in K level in the diseased group when compared to control one as showed in Table 5. These results agreed with those reported by Ali and Gomaa (2016).

Hypomagnesemia is often associated with hypocalcemia and decreased magnesium metabolism which may affect calcium hemostasis and metabolism. Where magnesium increases calcium release from the bone by displacing it from the hydration shell and by stimulation processes that involve the simultaneous catabolism of the mineral and matrix phase, thus, magnesium decrease would lead to a deficiency in calcium release from the bone and hence reduce its level (Randal et al., 2002).

Due and Care (1995) mentioned that in hypomagnesemia cases, there is a decrease in PTH formation, and hypomagnesemia cause resistance in target organs to the physiologic effects of PTH such as kidney, gut, and bone which leads to hypocalcemia and clinical disease. So, hypocalcemia is often associated with hypomagnesemia.

Low magnesium level decreases the activity of (25hydroxycholecalciferol-1-hydroxylase) enzyme which is responsible for vitamin D metabolism and Ca absorption (Matsuzaki et al., 2013).

There is no specific hormonal system able to regulate magnesium homeostasis (Urdaz et al., 2003). The kidney can excrete excess magnesium while it is unable to conserve enough magnesium systemically in case of deficiency. Therefore, the daily dietary magnesium intake is very important for animals, as there is only a small reserve of magnesium in the animals' body fluids, and bone metabolism is often insufficient to meet increased magnesium requirements when its demand increases (Odette, 2005). Furthermore, the feeding of the calves in this study on high protein content ration acts as a stressor where the availability and absorption of ingested magnesium decreased when rumen ammonia concentration increased. This stress may lead to clinical hypomagnesemia, as sympathetic nervous system activation causes an epinephrine release leading to a decrease in plasma magnesium level (Martens and Schweigel 2000).

Mild hyperkalemia lowered magnesium levels in CSF so, Mg influx into CSF may be inhibited by a high level of K in the blood. A stress reaction of animals involved the adrenal–glucocorticoid axis leading to increased circulating K level and lowered Mg transport across the choroidal plexus (Robson et al., 2004). Hypomagnesemia causes the resistance of target organs to the physiological effects of PTH that result in decreased bone resorption and calcium and P absorption from the digestive tract so, hypocalcemia and hypophosphatemia often accompany calf hypomagnesemia tetany.

Changes in liver and kidney enzymes and CPK levels due to hypomagnesemia

The result of the obtained study revealed that there was a significant increase in the level of ALT, AST, and ALP enzymes in the diseased group when compared to the control group. There was a significant decrease in the level of ALT, ALP, and AST in treated groups compared to the diseased group, as shown in Table 6.

Hepatocyte integrity can be monitored via AST and ALT levels (Bobe et al., 2004), where lower mineral concentration negatively affects ruminal micro-flora, which may lead to the production of toxic products (Wang et al., 2013). Increasing liver enzymes either directly through increased liver detoxification due to toxic load or indirectly because of increased oxidative stress (Maier et al. 2004, Guo et al., 2017). Another reason for the increase in the liver enzymes is a result of necrosis and muscular damage associated with a deficiency of magnesium, AST is released into general circulation in large concentrations and remains high for several days due to their normal physiological characteristics (Kumar et al., 1993 and Smith 1996).

Increasing the level of ALP may indicate that magnesium is an activator of some intracellular enzymes such as ALP (Sonnenwirth and Jarett, 1980).

These results were following that reported by (Ali and Gomaa, 2016).

The result of the obtained study revealed that there was a significant increase in the level of urea, creatinine, uric acid, and CPK in the diseased group when compared to control ones as shown in Table 7.

The results of urea and creatinine agreed with that reported by (Galbat et al. 2021).

The increased urea level might be attributed to the prerenal retention of nitrogenous wastes due to protein catabolism associated with anorexia and starvation. High urea levels may be attributed to renal dysfunction following calves' convulsive seizures (Kaneko et al.2008).

Creatine kinase is considered the most sensitive bioindicator of degenerative muscle disease in ruminant animals. The significant increase in serum CPK may be attributed to the muscular damage and necrosis in hypomagnesemia calves CPK is released into general circulation in large levels and remain high for days due to their normal physiological characteristics (Kumar et al,1993, smith 1996).

Changes in oxidants and antioxidants levels due to hypomagnesemia

There was a significant decrease in the level of GSH, SOD & TAC in the diseased group when compared to the control group while there was a significant increase in MDA in the diseased group when compared to the control one as shown in table 8.

These results agreed with that reported by El-Nile et al. (2018). The significant increase in MDA may be attributed to decreased ratio of Mg to Ca which stimulates the release of catecholamine from the adrenal glands that stimulates ROS production (Zheltovaa et al., 2016). While there was a significant decrease in the level of SOD as it was the first barrier against free radicals and reactive oxygen species scavengers in disease cases of animals (Celi, 2010).

SOD is an important factor in protection against harmful free radical activity and is considered the first defense mechanism against pro-oxidants so, its level decreased in case of stress or disease (Celi, 2010).

Hypomagnesaemia is a stressful condition that results in exhaustion and disturbance in the glutathione redox cycle and antioxidant enzymes (Rude, 1993, Abd El-Maksoud et al, 2012) because oxidative stress induced by hypomagnesemia includes disturbance between the pro-oxidant and antioxidant balance in favor to the former, which contributed to the developed pathologic effects observed in hypomagnesemia (Whang et al, 1994).

Changes in glucose, insulin, and cortisol levels due to hypomagnesemia

There was a significant decrease in the glucose, insulin, and cortisol levels in the diseased group when compared to the control one as shown in Table 9.

These results are in contrast with that reported by Ali and Goma (2016) and (El-Nile et al., 2018).

The significant glucose decrease in the present study may be attributed to the reduction in the food intake associated with hypomagnesemia (Hoff et al., 1993 and Attia, 1999) Moreover, Baig et al. (2012) attributed the decrease in serum glucose to the interrelationship between the metabolism of Mg and carbohydrate in diseased calves or inhibition of hepatic gluconeogenesis caused by elevated insulin.

The significant decrease in cortisol level may be due to stress reaction involving the adrenal–glucocorticoid axis that leads to an increase in the circulating K level and lowered Mg transport across the choroidal plexus, that was considered one of the main causes of this disease (Robnson et al., 2004).

Hypomagnesemia is generally associated with hypocalcemia that prevents insulin production, so its level decreased (Due and Care, 1995). Or may insulin level decrease due to an increase in the K level in diseased calves or maybe due to a decrease in glucose level and feed intake of diseased calves as insulin is associated with glucose metabolism. (Ali and Goma, 2016)

Changes in T_3, T_4, and PTH due to hypomagnesemia

There was a non-significant change in T_3 level between different groups, there was a significant increase in T_4 level in the diseased group when compared to control one and there was a significant decrease in parathyroid level in the diseased group when compared to control one as shown in table 10.

These results were in accordance with those reported by Ghanem (2013).

The main cause of hypomagnesemia in this study was the high content of protein in calves' ration. Serum thyroid hormones in calves fed high dietary protein concentration showed high thyroid status which may be due to the increased rate of oxidation and the continuous breakdown and formation of protein and fat (Ashmawy, 2015).

Hypomagnesemia leads to suboptimal conditions for enzyme reactions that need magnesium as a cofactor. Protein synthesis necessary to produce receptors for PTH in target cells may be curtailed during decreased magnesium levels. As well as decreased activity of adenylate cyclase which needs magnesium as a cofactor may disrupt the formation of cyclic AMP, the second messenger for PTH (Fontenot et al., 1989). Or decreased PTH formation level due to hypocalcemia (Martens and Schweigel, 2000).

Effect of treatment of hypomagnesemia calves with Mg oxide and Basil & Thyme

The result of the obtained study revealed that there was a significant increase in minerals contents, antioxidants, glucose, cortisol, insulin, and parathyroid levels in the treated group with Mg oxide and Basil and Thyme when compared to the diseased group as shown in Table 5, 8& 9. There was a non-significant change in T3 levels between different groups while, there was a significant decrease in liver and kidney enzymes, MDA, and T_4 levels in treated groups when compared to diseased ones as shown in Tables 6, 7& 10.

The result of increased mineral levels in treated groups with Mg oxide was in accordance with that reported by Helal et al. (2018). The result of Thyme disagreed with that reported by (Seirafy and Sobhanirad, 2017) due to the use of Mg oxide in the treatment of diseased animals so, the Mg level increased which was associated with an increase in Ca level.

Hypermagnesemia due to treatment with magnesium oxide causes the decrease in resistance of target organs to PTH that results in increased bone resorption and calcium and P absorption from the digestive tract so, hypercalcemia and hyperphosphatasemia accompanies calf treatment with magnesium (Martens and Schewel, 2000).

The result of decreased liver enzymes in Thyme treated group was in accordance with that reported by (Wafa et al., 2021 and Wafaa et al., 2022) and disagreed with that reported by (Ghoneem and Mahmoud, 2022).

This decrease in liver enzymes in the case of Basil and Thyme treated group may be attributed to the activity of Basil and Thyme in the elimination of the load on liver function due to its high antioxidative characteristics. Increasing serum Mg level by treating the animal with magnesium oxide also may directly affect the cellular redox state leading to a decrease in reactive oxygen species (ROS) production and reducing oxidative stress (Maier et al., 2004). This could be the reason for the decrease in hepatic enzymes.

The result of decreased level of kidney enzymes in the treated group with Thyme was in contrast with that reported by (Marwan and Mousa, 2021) and in accordance with that reported by (Baraz et al., 2021).

Ruminal ammonia-N is absorbed across the rumen wall into the portal blood and converted in the liver to urea. In the liver, synthesis of urea is performed from ammonia absorbed from the rumen so that the urea N blood level is highly correlated with the level of rumen ammonia-N (Davidson et al. 2003). Therefore, the decrease of urea level in our experiment in the treated Thyme group may be due to the impact of Thyme on hyper-ammonia-producing bacteria leading to a reduction in ammonia nitrogen production (Benchaar et al., 2008 and Seirafy and Sobhanirad, 2017).

Decreased CPK level may be due to the removal of animal convulsions and tremors due to the clinical signs' treatment with magnesium oxide or Basil and Thyme.

The result of increased TAC in the treated Thyme group was in accordance with that reported by (Miura et al., 2002). The main phenolic compounds of Thyme are ρ-cement monoterpene hydrocarbons (11.2%), carvacrol (3.5%), thymol (68.1%), and γ-terpinene (4.8%), which have a high significant antioxidant property (Rota et al., 2008) also, Basil has high antioxidizing characters related to its high major aroma compounds from its volatile extracts (Trevisan et al., 2006 and Soran et al., 2009).

The result of increased glucose and cortisol concentration agreed with that reported by Ghanem (2013) while the result of cortisol disagreed with that reported by (Helal et al. 2018).

Adequate level of Mg in the blood promotes synthesis of insulin leading to glucose uptake into the blood & its distribution to the functional cells (Vronese et al, 2016)

The increase in PTH level in the treated Thyme group came in accordance with that reported by Ghanem (2013). The difference between studies may be due to the variation of dosage or duration of treatment.

Evaluation of efficacy of treatment of hypomagnesemic calves with Mg salt and Basil and Thyme.

The result of the obtained study revealed that there was a significant decrease in ALT, AST, and glucose levels in the treated group with Basil & Thyme when compared to those treated with Mg salt and this may be attributed to the activity of Basil and Thyme in the elimination of the load on liver function due to its antioxidative properties while there was a significant decrease in cortisol in Mg salt treated group when compared to Basil & Thyme treated one at the level of p value $\leq$ 0.05 as showed in Table 11. These results indicated the efficacy of Basil and Thyme in improving the level of glucose and liver function tests in hypomagnesemic calves while the efficacy of Mg salt in the improvement of cortisol levels.

The result of the obtained study revealed that there was a non-significant change in some minerals (Ca, Mg, ionized Ca& Mg, K, P, and Na), ALP, kidney function test, CPK, oxidants, antioxidants, T_3, T_4, and PTH hormonal levels in treated group with Basil & Thyme when compared to those treated with Mg salt at the level of p value $\leq$ 0.05 as shown in Table 11.

Mg level in Mg oxide was 2.9 mg/dl this concentration in serum was high but within the normal range as that reported by (Hern´andez et al, 2020, Alcalde et al., 1999). An improvement in serum concentrations of Mg is due to the greater intake and solubility of the supplement, and more absorption by the ruminal papillae having less laxative effect (Odette, 2005, Schonewille et al., 2008).

Conclusion

Nervous manifestations are the most important critical signs of hypomagnesemia. Analysis of basil & thyme revealed their high content of Mg and improved their effect in the treatment of hypomagnesemia so, we suppose using them in treatment as Mg oxide.

The present work showed the biochemical alterations caused by hypomagnesemia in calves and the effect of using Mg oxide, Basil, and Thyme in the treatment of diseased calves. Calves are very important for the owner and the economy of the country so,

Prevention of them should be aimed at two major goals: continuous addition of adequate levels of magnesium in the ration and maximizing absorption of this essential mineral. Further research concerning the soil, plant, and animal, also factors affecting the availability of magnesium content for transport into the animal body, is very important for controlling this disease.

We recommend using Basil and Thyme as feed additives to the ration of calves.

More studies must be conducted on the use of Basil and Thyme for improving the immunity, health of the calves, and treatment of hypomagnesemia.

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