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BIOCHEMICAL STUDIES ON CHRONIC NON-INFECTIOUS, NON-PARASITIC DIARRHOEA IN CALVES REARED IN RECENTLY RECLAIMED AREAS IN SHARKIA GOVERNRATE

(With 4 Tables)

By

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دراسات بيوكيميائية على الاسهال المزمن الغير معدى والغير طفيلى فى العجول المرباه فى المناطق المستصلحة حديثا فى محافظة الشرقية

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استهدفت الدراسة فحص العجول التى تعانى من اسهال مزمن (مع استبعاد الاسهال البكتيرى أو الطفيلى) عن طريق دراسة المكونات البيوكيميائية لمصل ودم تلك الحيوانات بالاضافة الى تحليل بعض العناصر فى البرسيم الناتج من تلك الاراضى المستصلحة وربط نتائج هذه الدراسات والوقوف على أسباب وطرق تشخيص تلك الظاهره وعلاجها. وقد اجريت هذه الدراسة على عدد ٤٠ رأسا من العجول تراوحت اعمارها بين ٦ الى ٢٤ شهر وكانت تتغذى على البرسيم فقط طوال فترة موسم البرسيم وقد ظهرت عليها اسهال مزمن لم يستجيب للمضادات البكتيرية المعوية ولاللمضادات الطفيلية المعوية وضعف النمو وتغير فى لون الشعر وعدم القدرة على الحركة وبدأ عليها الهزال ولوحظ فى بعض منها تشوهات فى العظم مثل تضخم المفاصل. وقد اشتملت الدراسة ايضا عدد ١٠ عجول سليمة ظاهريا كانت تتغذى على برسيم مزروع فى القرى المجاورة (مناطق منزرعة قديما) واستخدمت كضوابط. وقد تم دراسة الاعراض الاكلينيكية واخذ عينات من دم ومصل تلك الحيوانات قبل وبعد العلاج. واسفرت نتائج التحليل عن انخفاض معنوى للعد الكلى لكرات الدم الحمراء وتركيز الهيموجلوبين وحجم الخلايا المضغوطة وارتفاع معنوى للعد الكلى لكرات الدم البيضاء فى الدم فى الحالات المريضة عند مقارنتها بالمجموعة الضابطة. وبالنسبة للتحليل البيوكيميائى لمصل الدم وجد انخفاض معنوى فى مستوى البروتين الكلى والالبيومين والجلوكوز والنحاس والحديد والفسفور والزنك والكالسيوم والصوديوم والبوتاسيوم. وكذلك

وجد ارتفاع معنوى فى مستوى عنصر المولبيدنيم فى مصل المجموعة المريضة بمقارنتها بالمجموعة الضابطة. أظهرت النتائج بالنسبة لتحليل البرسيم فى القرى المستصلحة حديثا وجود نقص معنوى فى مستوى النحاس والفسفور وزيادة نسبة كل من الكبريتات والمولبيدنيم ووجود الكالسيوم فى معدلة الطبيعى مقارنة بالبرسيم المنزرع فى القرى القديمة. وقد تم علاج حالات العجول المصابة بالاسهال المزمن بالمضادات البكتيرية والطفيلية ولم تستجب لهذا العلاج وبالتالى تم وضع مخلوط من كبريتات الزنك ٢٠٠ ملجم / كجم عليقة وكبريتات النحاس ٠,٧٥ جرام / كجم عليقة ثم أخذ ٢٥٠ جرام من هذا المخلوط ووضع فى عليقة كل عجل مريض يوميا لمدة ٥ اسابيع بالاضافة الى الحقن الوريدى بمحلول جلاسينات النحاس٦٠ ملجم لكل عجل جرعة واحدة. وبهذه المعاملة تم علاج حالات الاسهال الناتجة من نقص عناصر النحاس والزنك المزمن فى الغذاء مع توعية مالكى ومستصلحى هذه الاراضى الجديدة بضرورة اتباع الاسلوب السليم فى تغذية الحيوانات وعدم الاعتماد كلية على البرسيم والاعتماد على عليقة متزنة فى موسم البرسيم واضافة مخلوط الأملاح المعدنية والعناصر النادره خاصة النحاس والزنك الى هذه العلائق المقدمة للعجول المرباه فى المناطق المستصلحة حديثا.

SUMMARY

The aim of the present study was to investigate the effect of chronic non-infectious, non parasitic diarrhoea on blood hematological and biochemical parameters of calves excessively fed on Trifolium Alexandrium (barseem), during the green season in newly reclaimed area in Sharkia Governorate. Blood samples were collected from 40 diseased calves aged from 6-24 months showing signs of chronic diarrhoea, impaired growth, changes in hair colour, slow to move, poor body condition and skeletal abnormalities and not responded to local and systematic antibacterial and antiphrastic drugs. A number of 10 clinically healthy calves were selected at random basis from the adjacent villages (old agriculture area) used as control group. The hematological studies showed significant decrease ($p<0.001$) in TRBCs, Hb concentration, PCV and significant increase ($p<0.001$) in TWBCs in blood of diseased cases when compared with control group. Serum biochemical analysis revealed that calves with chronic diarrhoea exhibited marked decrease in total protein, albumin, globulins, glucose, copper, iron, inorganic phosphorus, zinc, calcium, sodium and potassium levels. Moreover, significant increase ($p<0.001$) in molybdenium levels was noticed when compared with apparent clinically healthy ones. Concerning the diet analysis, low copper and inorganic phosphorus contents were estimated, with higher concentration of sulphur and molybdenium were detected in this barseem grow in recently reclaimed

area compared with barseem grow in old villages. These results indicated primary phosphorus and secondary copper deficiency in the affected animals. After treatment with 250 gm of a mixture from (200 mg) zinc sulphate and (0.75 gm) copper sulphate per kilogram ration (orally) together with one dose of copper glycinate (60mg was given by IV injection) to the affected calf. The symptoms disappeared, included their health condition improved and the hematological as well as biochemical profile nearly returned to normal. So attention must be paid for non-infectious and non-parasitic causes of diarrhoea. Furthermore excessive feeding on barseem must be avoided and feeding balanced ration mixed with minerals, trace elements especially copper and zinc during nutrition of calves was recommended in the recently reclaimed areas of Sharkia Governorate.

Key words: *Diarrhoea, calves, blood, biochemical parameters.*

INTRDUCTION

The Egyptian government makes their best efforts to increase the agricultural and animal production through reclamation of new areas in different parts of the country to meet the rising demands of the increased human population. Soil, plant and animal interaction is generally recognized in relation to incidence of deficiency diseases. Diarrhoea remains one of the most important causes of calf morbidity and mortality in Egypt, (Kadry *et al.*, 2006). Diarrhoea is one of the common problems, treated under field conditions; the multiple etiological factors associated with diarrhoea often complicate the diagnosis. Persistent diarrhoea caused by secondary copper deficiency with the signs of watery, yellow, greenish to blackish feces with an offensive odour, in some cases within 8-10days of feeding on affected pastures, (Radostits *et al.*, 2000). Copper deficiency is a wide spread economic problem in ruminants especially in cattle, (Wikse *et al.*, 1992). Copper deficiency in ruminants can occur as a primary deficiency, where copper intake is inadequate or as a secondary deficiency, where by other factors in the diet interfere with the absorption or metabolism of copper, (Gengelbach *et al.*, 1994). Copper bioavailability in ruminant diets is particularly low when molybdenum and sulpher are present in moderate to high concentrations, which results in the formation of highly antagonistic thiomolybdates, (Suttle, 1991). Copper deficiency in calves may result in decreased growth, anemia, severe diarrhoea, change in hair colour, heart

failure, weak bones and reduced reproductive efficiency, (David *et al.*, 1999). Zinc and copper inhibit the absorption of each other, where zinc would interfere with copper metabolism because it had the same chemical parameter. (Mohga, 2000). In view of the above speculation, the present investigation was carried out to study the hematological and serum biochemical changes in calves suffering from chronic non-infectious, non parasitic diarrhea with history of excessive prolonged feeding on Trifolium alexandrium (barseem) in newly reclaimed areas of Egypt and failure of antibacterial and antiparasitic treatment to cure the diarrheatic cases. This may help in the solving this problem, as well as to reach ideal way for treatment and will contribute to the understanding of aetiopathology of chronic diarrhoea. Suggestive measures for the prevention and control of disease in calves was also aimed.

MATERIALS and METHODS

1 - Animals:

The present investigation was carried out on 40 calves of both sexes aged (6-24 months) with history of excessive prolonged feeding on Trifolium alexandrium (barseem), calves were randomly selected from field cases that raised in newly reclaimed area at north and south El-Hosynia and the villages surrounding at Sharkia Province, (Egypt). The disease was clinically diagnosed on two bases, the specific signs such as impaired growth, lameness, stiffness, severe diarrhoea, achromotrichia, heart failure and mild anemia, also the diagnostic treatment not respond to antibacterial and antiparasitic therapy. The control group included 10 clinically healthy calves of both sexes and at the same age selected from the adjacent villages (old agriculture area) with history of excessive prolonged feeding on barseem and without signs of diarrhea.

2 - Sampling:

Two blood samples were collected from each animal in both diseased (2-weeks post antibacterial and antiparasitic treatment) and control groups before and after one month post mineral treatments.

A- The first blood samples were collected in heparinized tube (with heparin anticoagulant) for the estimation of erythrocytes (TRBCs), leukocytes (TWBCs) counts, packed cell volume (PCV) and hemoglobin (Hb) concentration (Coles, 1986). Red blood cell indices were also calculated.

B- The second blood samples were collected without anticoagulant for separation of serum for determination of total proteins level (Doumas, 1974), albumin level (Drupt, 1974), and serum globulins levels were calculated as difference between total protein and albumin, serum glucose level (Trinder, 1969), serum calcium level (Gindler and King, 1972), serum inorganic phosphorus level (Morinal and Prox, 1973), serum sodium and potassium levels (Hawk, 1965) by using a flame-photometer (Corning model AVL 988-3, made in U.S.A.). Serum traces elements (iron, zinc, copper and molybdenum) levels were estimated by atomic absorption spectrophotometer according to Cowell (1973).

C- Faecal samples:

Faecal sample was collected from each examined animal for detection of any internal parasite according to Soulsby (1986).

D- Skin scraping:

Skin scraping was carried out for detection of dermatophytes and metazoan parasites according to Coles (1986).

F- Diet samples:

Five samples of barseem from recently reclaimed area and from old villages were collected and analysed for copper, molybdenum, phosphorus, calcium and sulphur estimation according to A.O.A.C. (1975).

3 - Treatment trials:

The principal line for treatment was based on a diagnostic treatment by antibacterials and antiparasitic to excluding the infectious and the parasitic cases of diarrhoea, correction of the feeding system used for the affected animals for compensation of the recorded deficiency status.

After excluding the parasitic and infectious cases of diarrhoea (15 days post treatment) we could follow such treatment program:

1- Correction of feeding system: avoiding the excessive feeding on barseem with preparation balanced ration during the green season.
2- The diseased animals were given 250 gm of mineral mixture containing zinc sulphate (200 mg/kg feed), copper sulphate (0.75 gm/kg feed) daily to each calf for 5 weeks (Radostits *et al.*, 2000).
3- Copper glycinate (60mg for calf) one dose intravenously injected to maintain adequate copper levels for about 60-90 days.
4- Statistical analysis: The obtained data was analyzed using the student's t-test (Snedecor and Cochran, 1982).

RESULTS

Table 1: Haematological variables (mean ± SE) in blood of healthy calves and those affected with chronic diarrhoea before and one month after treatment.

Items / Parameters		Control healthy calves	Chronic diarrhea affected	Post treatment
TRBCs	($x10^6$cum)	8.23 ± 0.26	5.67 ± 0.19***	7.70 ± 0.12
Hb	(gm/dl)	11.77 ± 0.32	8.56 ± 0.16***	11.49 ± 0.17
PCV	(%)	39.19 ± 0.42	33.70 ± 0.40***	39.07 ± 0.23
MCV	(cuu)	46.17 ± 0.67	59.17 ± 1.70***	47.68 ± 0.44
MCH	(ug)	15.56 ± 0.33	18.49 ± 0.31***	16.28 ± 0.16
MCHC	(gm/dl)	32.53 ± 0.47	27.51 ± 0.39***	31.82 ± 0.20
TWBCs	($x10^3$ cum)	8.45 ± 0.27	10.75 ± 0.15***	8.60 ± 0.15

N.B. NS: non significant ***: very highly significant at ($p<0.001$)

Table 2: Biochemical values (mean ± SE) in serum of healthy calves and those affected with chronic diarrhoea before and one month after treatment.

Items / Parameters		Control (healthy calves)	Chronic diarrhoea (affected calves)	One month Post treatment
T.Proteins	(gm/dl)	7.58 ± 0.12	5.92 ± 0.14***	7.44 ± 0.08 NS
Albumin	(gm/dl)	4.08 ± 0.17	3.06 ± 0.08***	4.15 ± 0.07 NS
Globulins	(gm/dl)	3.52 ± 0.13	2.87 ± 0.16**	3.28 ± 0.09 NS
Glucose	(mg/dl)	72.26 ± 1.57	52.29 ± 1.02***	70.36 ± 0.95 NS

N.B. **: NS: non significant highly significant at ($p<0.01$)
***: very highly significant at ($p<0.001$)

Table 3: Blood serum macro and micro element levels (mean ± SE) in healthy calves and those affected with chronic diarrhoea before and one month after treatment.

Items / Parameters	Control (healthy calves)	Chronic diarrhoea (affected calves)	One month Post treatment
Copper (ug/dl)	74.43 ± 1.05	40.28 ± 2.55***	63.70 ± 1.05***
Iron (ug/L)	161.66 ± 3.91	122.48 ± 3.05***	163.72 ± 1.93 NS
Molybdenum (ug/dl)	55.04 ± 0.47	74.68 ± 1.78***	53.68 ± 0.64 NS
Phosphorus (mg/dl)	6.29 ± 0.18	4.20 ± 0.12***	6.36 ± 0.07 NS
Zinc (ug/L)	170.22 ± 2.46	103.22 ± 4.68***	165.18 ± 1.58 NS
Calcium (mg/dl)	10.65 ± 0.20	8.64 ± 0.17***	10.30 ± 0.12 NS
Sodium (mmol/L)	142.21 ± 1.20	121.58 ± 2.05***	139.97 ± 0.94 NS
Potassium (mmol/L)	5.43 ± 0.07	4.23 ± 0.09***	5.21 ± 0.06*

N.B. NS: non significant *: significant at ($p<0.05$
***: very highly significant at ($p<0.001$)

Table 4: Copper, molybdenum, phosphorus, sulphur and calcium contents of barseem from newly reclaimed area and old agricultural area (old villages) on dry matter basis.

Parameter / Items	DM basis				
	Copper mg/kg	Molybdenum mg/kg	Phosphorus %	Sulphur %	Calcium %
Barseem from old villages (Control)	7.04 ± 0.33	0.86 ± 0.02	0.29 ± 0.02	0.22 ± 0.24	3.50 ± 0.10
Barseem from newly reclaimed area	5.61 ± 0.17*	4.9 ± 0.10***	0.18 ± 0.03*	0.59± 0.03***	3.43± 0.14 NS

N.B. NS: non significant *: significant at ($p<0.05$)
***: very highly significant at ($p<0.001$)

DISCUSSION

1 - Clinical findings:

Clinical survey was conducted on 40 calves suffering from chronic diarrhoea. The main clinical signs were impaired growth, anaemia, poor body condition, changes in hair colour, heart failure, skeletal abnormalities, lameness and persistent diarrhoea with the passage of watery, yellow – green to black faeces with an offensive odour occurs. The faeces are released without effort, often without lifting the tail. These symptoms were recorded previously by Maas and Bradford (1990); Murray *et al.* (1999); Radostits *et al.* (2000), who recorded also that, the diarrhoea is may usually the only a major clinical finding in secondary copper deficiency associated with molybdenosis. While Asma (1997), stated that the impaired growth could be attributed to the decreased level of serum copper due to poor quality roughage or inadequate concentrations of minerals. Also copper deficiency impairs the conversion of food into energy and therefore copper deficient calves showed decreased appetite and poor body condition (George and Fisher, 2008).

2 - Laboratory findings:

Analysis of barseem samples collected from recently reclaimed areas and old agricultural area (old villages) revealed that the concentration of copper, molybdenum, phosphorus, calcium and sulphur content were suggestive of primary significant phosphorus and secondary copper deficiencies (Table 4). The low copper levels in barseem from recently reclaimed area are generally considered adequate for calves' requirement unless complicating factors cause secondary deficiency (Underwood and Suttle, 1999). It would appear that copper deficiency is caused by the relatively high concentration of molybdenum and sulphur in barseem of recently reclaimed area, which effectively reduce the availability of dietary copper to calves. As high levels of molybdenum in the diet can bind with copper in reticulo-rumen, forming an insoluble copper molybdate complex. Similarly, in the presence of dietary sulphates, copper binds to sulphur forming a non absorbable copper sulphate complex, this reduces copper absorption and if persist for a period it creates a secondary state of deficiency (Gooneratne *et al.*, 1989). However, Mullis *et al.* (2003), recorded that a diet containing 6.4 mg copper / kg of dry matter did not meet the copper requirements of growing heifers. Also barseem analysis revealed phosphorus deficiency

in recently reclaimed area which may be attributed to the low phosphorus content in the parent rock from which the soils are derived (Radostits *et al.*, 2000). On the other hand the excess of molybdenum in barseem grown on newly reclaimed area reduces phosphorus content in the body by interfering with its absorption from the gastro-intestinal tract and increasing phosphorus elimination through urine (Dhillon *et al.*, 1972). Concerning the laboratory studies on haematological profile (table 1), macrocytic hypochromic anaemia was evident. There was a very highly significant decrease ($p \leq 0.001$) in TRBCs count, Hb concentration and PCV % values in diseased calves in compared to healthy control ones. The reduction in these blood parameters may be due to iron, copper and / or protein deficiences (Osman *et al.*, 1991), as copper acts as a catalyst in the production of haemoglobin and facilitates the absorption of iron and formation Hb of new RBCs. The larger MCV with lowered MCHC indicate that anaemia is of macrocytic hypochromic type which may be due to malnutrition and / or malabsorption (Abdel-Maksoud and Abdel-Raoef, 1998). The explanation of the anaemic condition in diseased calves is a reasonable finding in copper, iron and zinc deficiency. Copper is necessary for the reutilization of iron liberated from normal breakdown of Hb by the activation of ferroxidase enzyme (Radostits *et al.*, 2000). Concerning the total leucocytic count in the present study, results in (Table 1). showed very highly significant leucocytosis ($p \leq 0.001$) in diseased calves than that of control ones. Leucocytosis in diseased calves (the increased TWBCs) might be due to inflammatory reactions occurred in the digestive tract of diseased calves due to the presence of diarrhoea. These results are in agreement with those mentioned by Radostits *et al.* (2000) and Abdel-Maksoud and Abdel-Raoef, (1998). In present study, the highly significant decrease ($p \leq 0.001$) in serum total proteins, albumin and globulins level in diseased calves presented in (table 2), was nearly in accordance to those observed by Mohga (2000); Tawfik *et al.* (2004); Shalaby *et al.* (2008), who stated that the decreased values of serum total proteins are the possible explanation of drastic reduction that could be due to stress factors, for which the animal is exposed to general unthriftness which may affect worsly the hepatic parenchyma resulting in the failure of protein synthesis. The significant decrease in albumin concentration might be due to either decreased food intake or albumin loss as a result of increased capillary permeability in copper deficient animal due to abnormalities in the blood vessel wall elastin and collagen structures (O Dell, 1976 and Rucker and Tinker, 1977). As present in

(Table 2) hypoglycaemia ($p \leq 0.001$) noticed in calves suffering from chronic diarrhoea may be due to loss appetite or depressed metabolic processes which consequently reflected upon glucose metabolism and on its levels. Rather similar results were previously reported by (Coles, 1986; Tawfik *et al.*, 2004). The obtained data concerning serum copper levels in calves suffering from chronic diarrhoea were below normal ($p \leq 0.001$) (Table, 3). Underwood, (1977) indicates that plasma copper concentration below 50 ug/dl is indicative of depleted liver copper stores and copper deficiency in animal. Calves born to copper depleted cows fail to receive adequate copper through placental transfer or through the colostrum. Copper deficiency in this study may be occurring due to low levels of copper in the forage, low levels of copper in the mineral supplement or poor mineral supplement intake and or high levels of antagonistic minerals in the diet such as sulphur, iron and molybdenum. Nearly similar data were recorded by Mullis *et al.* (2003); Sallam and Abdel Maghney, (2009). A highly significant decrease ($p \leq 0.001$) (Table 3), was existed in blood serum iron level in calves suffering from chronic diarrhoea if compared with that of clinical healthy ones. The results were recorded by Georgievskil, (1982), that proved that there are direct interaction between copper and iron as a joint participation of iron and copper in the formation of haemoglobin. Copper is essential for the incorporation of iron in the heme molecule and its deficiency inhibits the transport of iron by decreasing the levels of ceruloplasmin ferroxidase leading to decreased heme production and haemoglobin synthesis (Gregg *et al.*, 2002). On the other hand results shown in (Table 3) cleared a highly significant increase ($p \leq 0.001$) serum molybdenum level accompanied by phosphorus and copper deficiency in calves suffering from chronic diarrhoea, if compared with that of clinically healthy ones. Similar results were obtained by Daved *et al.* (1999); Shalaby *et al.* (2008). Such elevation can be considered as a result of natural or anthropogenic (eg. Mining manufacturing) processes. Molybdenum may become sufficiently concentrated in soil and vegetation (barseem) to cause molybdenosis in calves (Raisbeck *et al.*, 2006). Regarding to serum inorganic phosphorus level, the obtained results shown in (Table, 3) indicated highly significant decrease ($p \leq 0.001$) of the diseased calves in this study. This result is in agreement with that reported by Emam *et al.* (2005); Akhtar *et al.* (2007); Shalaby *et al.* (2008), who mentioned that the decreased phosphorus level may be due to long feeding on barseem with low level in phosphorus content. Moreover, soils in the newly reclaimed area have high molybdenum content; consequently the

fodders in particular barseem grown on such soils will have high molybdenum content. The excess of this element reduces phosphorus level in the body by interfering with its absorption from gastro-intestinal tract and increasing phosphorus elimination through urine (Dhillon *et al.*, 1972). Deficiency of zinc in calves ($p \leq 0.001$) shown in (Table, 3), are not uncommon in the post weaning period and may be attributed not only to inadequate diet but also to the fact that calves do not adjust themselves to dry feed effectively for up to several weeks post weaning (Underwood, 1977; Sadiek *et al.*, 1994; Radostits *et al.*, 2000). Regarding results of biochemical profile of some mineral levels (Table 3), obtained data revealed highly significant decrease in serum calcium, sodium and potassium levels between diarrhoeic calves and healthy ones. These results came in agreement with that of Mckcown, (1984); Nasser *et al.* (2000); Tawfik, (2000), who pointed out that its depletation most commonly develops as the result of gastro-intestinal losses through diarrhoea. Moreover, the amount of sodium loss in faeces of diarrhoeic calves is 27.2 times more than normal calves (Radostits *et al.*, 2000).

After treatment:

An observable advance in both clinical and laboratory results of calves suffered from chronic diarrhoea after correction of the diet system was achieved through avoiding the excessive feeding on barseem and providing the animal with balanced ration. The diseased cases were treated by 250 gm of mineral mixture containing zinc sulphate (200 mg / kg feed), copper sulphate (0.75 gm / kg feed) daily offered to each calf for 5 weeks (Radostits et al., 2000),together with one dose of copper glycinate (60 mg for calf) was intravenously injected once.

It could be concluded that chronic diarrhoea noticed in calves fed excessively on barseem from recently reclaimed area could be attributed to copper deficiency which plays a key role in causing chronic diarrhoea in calves. The affected calves showed hypo copraemia and reduce total erythrocytic counts and this in turn reflects the disturbance in general healthy condition of the affected animals. So attention must be to careful early clinical and laboratory diagnosis of diseased animals followed by therapeutic plan with good management. Furthermore excessive feeding on barseem must be avoided and feeding balanced ration during the green season is also advised.

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