Dpt. of Animal Medicine,
Fac. of Vet. Med., Assiut University, Assiut, Egypt.

SOME TRACE ELEMENTS AND ANTIOXIDANTS PROFILE IN ILL-THRIFT FRIESIAN CALVES

(With 4 Tables)

By

N.M. AREF; M.R ABD ELLAH; G.F. KHAMIS*;
M. ABDEL-MOAETY** and A.A. AAMER

*Animal Health Research Institute, Assiut, Egypt.
**Dept. of Biochemistry, Fac. of Medicine, Sohag University, Sohag, Egypt.

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مستويات بعض العناصر النادرة ومضادات الأكسدة في العجول الفريزيان ضعيفة النمو

نصر الدين محمد عارف ، محمود رشدي عبد اللاه ، جابر فرغلى خميس ، محمد عبد المعطي ، أحمد عبد الفتاح عامر

تمثل العناصر النادرة مكون رئيسي من مكونات الدم في الحيوانات المجترة. فإنها تعمل في جميع جوانب الحياة، من إنتاج الطاقة ، توازن الدم ، هضم وانتاج. وبالتالي فإن نقص هذة العناصر غالباً ما يؤدي إلى ضعف النمو (اعتلال الادخار) وقلة الخصوبة. أستهدفت الدراسة تقييم تركيزات بعض العناصر النادرة ومدي نشاط مضادات الاكسدة الكلية في العجول البقري والتي تعاني من ضعف النمو واعتلال الصحة. لتنفيذ الدراسة تم أختيار عشرون عجل فريزيان. تم تقسيم العجول البقري العشرون الي مجموعتين: عشرة في المجموعة ١ (ضابطة) وعشرة في المجموعة ٢، تعاني من ضعف النمو. أجرى تقييم لصورة الدم الكاملة ، العناصر النادرة ومدي نشاط مضادات الاكسدة الكلية. وأظهرت النتائج انخفاضا معنوياً ($P<0.01$) في معدلات كلا من النحاس والكوبالت في مصل الدم مصحوبة بصورة الانيميا ذات الكريات الصغيرة microcytic normochromic في العجول المريضة بالمقارنة بمثيلاتها السليمة وصاحب هذا الانخفاض انخفاضا معنوياً ($P<0.01$) في نشاط مضادات الاكسدة الكلية. وأوضحت الدراسة أن ضعف النمو (اعتلال الادخار) في العجول البقري تعزى إلى حد كبير إلى نقص العناصر النادرة– وخاصة في مستويات النحاس والكوبالت– والتي قد تسبب بدورها انخفاض في نشاط مضادات الاكسدة الكلية.

SUMMARY

Trace elements are the basic components of enzymes and co-enzymes in the biochemistry of ruminants. They function in all aspects of life, from

energy production, blood homeostasis, to digestion and reproduction. Thus, their deficiency often leads to sub-optimal growth (ill-thrift) and infertility. The objectives of this study were to evaluate some trace elements concentrations and the activity of antioxidant systems in ill-thrift calves. Twenty calves were divided in two groups: ten in group 1 (control) and ten in group 2, which showed sub-optimal growth. Complete blood picture, trace elements profile and total antioxidants capacity were evaluated. Results showed a significant reduction ($P \leq 0.01$) in the blood serum concentration of copper and cobalt associated with microcytic normochromic anemia. Also a significant reduction ($P \leq 0.01$) in the activity of total antioxidants was evident in ill-thrift calves. In conclusion, a state of sub-optimal growth (ill-thrift) in calves was largely attributed to trace element deficiency, in particular cupper and cobalt deficiency that may cause reduction in the total antioxidant capacity, with a lower ability to reduce oxidative compounds.

Key Words: *Trace elements, antioxidants, ill-thrift calf.*

INTRODUCTION

Calf ill-thrift is a vaguely defined condition with a variety of causes however ill-thrift and suboptimal growth are terms often used interchangeably (Radostits *et al.*, 2000). Failure to gain weight is the main feature of this condition and chief complain of livestock producers. It has a drastic economic impact on livestock production as it affects animal's rate of body weight gain, marketing, day to the first calving, herd survivorship and future productivity (Radostits, *et al.*, 1994; Underwood & Suttle, 1999). While the most likely cause of this condition is the disturbance of metabolism secondary to micronutrients: copper, cobalt, selenium, zinc and iron, imbalances; the pathophysiological basis of such condition is still intricate (Mills, 1983). However, it is well established that trace elements are involved as component parts of many tissues and function as cofactors, enzymes or stabilizers of secondary molecular structure (Valee and Wacker, 1976, George and Fisher, 2008) and their deficiency leads to a wide variety of metabolic disturbances and pathological consequences (Mills, 1985). A number of stre: factors including environmental and managemental variables such as hot climatic condition, type of soil and weaning practices have also been reported to be contributing factors to calf ill-

thrift (Reid and Howath, 1980; Scibilia, *et al.*, 1987). Stress of trace elements deficiencies and hot climatic condition generally increases the production of free radicals, leading to oxidative stress (Elsayed, 2001; Saleh *et al.*, 2008) which has a negative impact on the calves live weight, mortality and health.

The present study was undertaken to describe the clinical course of the condition and evaluate some trace elements status and antioxidant profile in ill-thrift calves. The free radical defense system in ill-thrift calves was assessed by measuring total antioxidant capacity (TAC) which considers the cumulative effect of all antioxidants present in blood and body fluids (Miller and Rice-Evans, 1994) not just the antioxidant capacity of a single compound.

MATERIALS and METHODS

The study area: A dairy farm is located in an arid tropical area underneath the eastern mountain of Assiut governorate- Bani Qura-Quesia, Assiut- Egypt. No surface water and rainfall is negligible. Calves in this area reared under unsatisfactory standards of animal management and feeding however, strategy for combating parasitic infestation was applied by giving regular dose of anti-helminthes drugs.

Animals: The study was conducted on 20 Friesian calves, 6-8 months of age (7.40 ± 0.65) in the period of April-June, 2009. Ten calves showed general weakness, poor growth rate (way less than 0.8 kg/day), and anemia with no apparent diseased condition. Failure to gain weight was the chief complain (B.W/kg =114.5 ± 11.41). The other ten calves were clinically healthy and used as a control. They showed normal growth rate (≥ 0.8 kg/day) and body weight (B.W/kg = 178.7 ± 14.76) under farm condition.

Blood and fecal sampling:

Whole blood samples: 10 ml of blood was drawn from the jugular vein of each calf in clean centrifuge tubes containing Na_2-EDTA as an anticoagulant for complete blood picture (CBC) evaluation.

Serum sample: 10 ml of blood was drawn from the jugular vein of each calf in clean centrifuge tubes without anticoagulant. Sera were separated by centrifugation and stored at -20°C until used (Coles 1986). The serum was clear and free from haemolysis. The blood serum used for measuring serum total proteins, albumin, liver enzymes, some trace elements (Cu, Co, Fe and Zn), hydrogen peroxide and total antioxidants capacity.

Faecal samples: Representative samples (10 samples, each of ~100 g) of feces were collected from each animal and stored in air-tight containers for subsequent macroscopic and parasitological analysis by the standard flotation sedimentation technique (Coles, 1986).

Hematological and biochemical analysis:

Total red blood cell count (TRBCs T/l), hemoglobin concentration (Hb g/l), packed cell volume (PCV%), mean corpuscular volume (MCV fl) and mean corpuscular hemoglobin (MCH pg), were determined using a fully automated blood cell counter machine, Abbott cell-dyne 1700, in the department of Animal Medicine- Faculty of Veterinary Medicine- Assiut University, Assiut-Egypt.

Trace elements determination: Concentrations of Cu, Co, Fe, and Zn (µg/dl) in the serum were determined with an atomic absorption spectrophotometer (GBC 906 AA; GBC Scientific Equipment, Australia) after wet ashing in perchloric, and nitric acids in Soil and Water department-Faculty of Agriculture- Assiut University, Assiut-Egypt.

Liver function tests:

Measurements of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin (ALB) and total protein (TP) were carried out using reagent test kits supplied commercially by Boehringer Mannheim GmbH Diagnostica. Spectrophotometeric assay was performed using Phillips Pye Unicam spectrophotometer (U.V. visible) Mod 800 in the department of Animal Medicine- Faculty of Veterinary Medicine- Assiut University, Assiut-Egypt.

Antioxidant profile:

Total Antioxidant Capacity (TAC):

TAC was measured by reaction kinetics according to Miller *et al.* (1993 & 1996). Briefly, water blank, standard plasma or standard (hydroxyl-tetramethylchroman carboxylic acid) was added to chromogen 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and horse radish peroxidase and read initial at OD_{600} nm (A1) and 3 minutes after adding H_2O_2 (A2). ΔA minute calculated by subtracting A1 from A2 and dividing the result by 3. Standard curve was done by construct difference between ΔA minute for standards and that of blank against their concentrations. The total plasma antioxidant capacity was expressed in µg/mL.

Plasma inorganic hydrogen peroxide (H_2O_2): Plasma H_2O_2 was measured by colormeteric method depending on ability of peroxidase to reduce H_2O_2 at the expense of perchloric acid ($HClO_4$) (Bernt and

Bergmeyer 1974). Briefly, Equal volume of perchloric acid (1.0 N) mixed with deprotinized plasma, centrifuge and 10 µl of supernatant added to peroxidase enzyme mixed with phosphate buffer then had read at OD_{600} nm. Peroxide content was calculated from the standard curve and expressed in µM.

The antioxidant profile was assessed in the department of Biochemistry- Faculty of Medicine- Sohag University, Sohag- Egypt.

Statistical analysis:

Data were analyzed using the packaged SPSS program for windows version 10.0.1 (SPSS Inc., Chicago, IL). Data were presented as mean ± standard deviation (SD). Differences between groups were determined by the one-way analysis of variance (ANOVA). Significance level was set at $P \leq 0.01$ and $P \leq 0.05$.

RESULTS

Clinical course:

No specific signs of ill-thrift were observed. Poor growth, wasting and weakness were the only obvious clinical signs. Pica, poor coat and hair condition were likely noticed. There was marked pallor of the mucous membranes and examined calves were easily fatigued. No systemic reaction, however extracardiac murmurs were evident on through clinical examination with obvious negative jugular pulsation.

Fecal and hematological analysis:

Analysis of fecal sample by standard sedimentation floatation technique revealed no evidence of parasitic infestation. The hematological indices including RBC, Hb and PCV were significantly decrease ($p< 0.01$) in ill-thrift calves compared with controls. The MCV and MCH values revealed a microcytic normochromic type of anemia in ill-thrift calves in comparison with the control values (Table 1).

Trace elements concentrations and biochemical results:

Result of liver enzymes showed no significant differences. Serum albumen, total protein and liver enzymes, AST & ALT, showed no significant change in comparison with the control group (Table 2). A significant decrease ($p<0.01$) in the concentration of serum cupper and cobalt and marginally deficiency in iron were evident while no change in the level of zinc (Table 3).

Hydrogen peroxide and total antioxidants capacity:

The study showed substantial increasing ($p \leq 0.05$) of H_2O_2 levels with significant reduction ($p \leq 0.01$) in total antioxidants capacity in ill-thrift calves in comparison with the control group (Table 4).

Table 1: Mean values (±SD) of hematological indices in control and ill-thrift calves

Parameter	Control	Ill-thrift calves
RBCs (T/l)	7.71 ± 0.61	4.92 ± 0.16*
Hb (g/dl)	11.74 ± 0.43	10.16 ±0.33*
PCV (%)	35.80 ± 0.96	23.28 ± 2.90*
MCV (fl)	48.32 ± 3.02	36.65 ± 2.19*
MCH (pg)	16.12±3.5	20.77 ± 2.38

* Significant difference at 0.05

Table 2: Mean values (±SD) of liver function tests in control and ill-thrift calves

Parameter	Control	Ill-thrift calves
TP (g/dl)	6.69 ± 0.53	6.15 ± 0.92
Albumin (g/dl)	3.49 ± 0.39	3.62 ± 0.59
AST (IU/l)	30.21 ± 2.04	23.5 ± 1.71
ALT (IU/l)	13.25 ± 1.00	14.47 ± 0.43

Table 3: Mean values (±SD) of serum micronutrients concentrations in control and ill-thrift calves

Parameter	Control	Ill-thrift calves
sCu (µg/dl)	98.34 ± 2.14	60.00 ± 11.57**
sCo (µg/dl)	0.54 ± 0.003	Undetectable
sZn (µg/dl)	98.33 ± 0.61	84.00 ± 18.00
sFe (µg/dl)	198.12 ± 53.65	162.2 ± 27.26

** Significant difference at 0.01

Table 4: Mean values (±SD) of total antioxidant profile in control and ill-thrift calves

Parameter	Control	Ill-thrift calves
H_2O_2 (µM)	368.2 ± 75.39	513.60 ± 83.07*
TAC (µg/ml)	2.58 ± 0.44	1.1 ± 0.32**

*Significant difference at 0.05
** Significant difference at 0.01

DISCUSSION

Trace elements are essential for normal growth and their study has evolved from recognition of their vital role in cell metabolism. There has been special interest in effects of trace elements deficiencies on physiological functions in general and in normal growth rate and oxidative process in particular. In this respect, screening for some trace elements concentrations and total antioxidants activity were evaluated in ill-thrift calves.

Our data suggested that a concomitant ill-thrift in cattle is largely due to significant reduction in serum cupper, cobalt and marginally deficiency in serum iron concentrations. It is well established that lack of Cu and Co impair the conversion of food into energy and therefore Cu and Co- deficient calves go off their diets and begin to waste away (George and Fisher 2008). Microcytic anemia was evident and represents an additional complicating factor for suboptimal growth. There was significant reduction in the size of RBCs (MCV) and significant decrease in the total RBCs count. Although the total Hb concentration (Hb g/l) showed significant reduction, the mean concentration of Hb within the small sized- RBCs (MCH) showed no change. The serum level of Fe was not significantly, rather marginally, decrease in ill-thrift calves to induce hypochromic anemia. It is also believed that suboptimal growth in calves is associated with reduction of antioxidants activity resulting from cupper deficiency. This reduction of antioxidants activity in the hypocupremic calves may allow reactive oxygen species (ROS) such as superoxide ($O_2^{\cdot -}$) to accumulate beyond their capacity to an extent that oxidative damage may occur. A significant increase ($P \leq 0.05$) of serum hydrogen peroxide (H_2O_2) in Cu deficient ill-thrift calf strongly support this assumption and in agreement with Fernandez-Urrusumo *et al.* (1997). In such case, it may be hypothesized that a state of negative control on total antioxidant capacity occurs due to overwhelmed antioxidant system secondary to cupper deficiency, release of lipid peroxide, and consuming of total antioxidants capacity by ROS. These may explain why the activity of TAC decreased. Previous studies showed a tendency for leukocytes to release greater amounts of $O_2^{\cdot -}$ in hypocupremic animals (Jones and Suttle, 1981). For instance, 58% increase in $O_2^{\cdot -}$ was reported in hypocupremic rat tissues (Lynch *et al.*, 1997). Also, elevated $O_2^{\cdot -}$ and diversion of nitric oxide (NO) by $O_2^{\cdot -}$ to peroxynitrite (ONOO−), a potent oxidizing agent, were found in the Cu deficient rat embryos (Hawk *et al.*, 2003;

Beckers-Trapp *et al.*, 2006). It is well established that copper is an essential micronutrient, occurs in certain oxidase and plays a key role in body defense against free radicals (Uriu-Adams and Keen, 2005) which cause damage to cell membranes with subsequent alteration in natural cellular metabolism.

In short, trace elements deficiencies, cupper and cobalt in particular, have been incriminated as a major contributing factor to suboptimal growth and oxidative inducing condition in ill-thrift calves.

CONCLUSION

What is entirely certain is that deficiencies of one or more trace elements markedly reduce the live body weight with subsequent reduction in the profitability of a livestock agricultural system. The results may also suggest that the poor nutritional status caused modifications to the antioxidant systems, with a lower ability to reduce oxidative compounds. Further controlled studied are needed to investigate the association between trace elements and total antioxidants activities on larger sample size.

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