Clinical hematological, biochemical and pathological studies on mineral deficiency with special reference to hypozincemia in Egyptian buffaloes

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Abstract

Hematological, pathological and some biochemical parameters have been studied in buffalo calves affected naturally with hypozincemiain private farm in Marsamatroh., among these animals,(10 buffalo calves were 15-20 months old were examined). Five clinical healthy buffalo calves in the same age were used as control. Affected buffalo calves showed signs of alopecia indifferent body regions, abnormal skin (rough, thickened, wrinkled, cracked with dandruff).paleness of mucous membranes, intermittent diarrhea, loss of appetite and decreased growth rate, swelling of joints and stiff gait and pica. No significant difference has been detected in body temperature, whereas respiratory and heart rates were significantly increased in affected buffalo calves in comparison with control. Statistical analysis showed significant decrease in the total erythrocytes (RBCs), hemoglobin (HB) and packed cell volume (PCV) in diseased buffalo calves and macrocytic normochromic type of anemia was found. The biochemical results revealed highlysignificant decrease in serum zinc and some minerals (manganese, copper, iron and electrolytes) and increase in AST, ALT ,Urea, Creatinin, LDH and creatine kinase (CK) in diseased buffalo calves. Microscopic lesions of the skin of zinc deficient buffalo calves were in the form of epidermal hyperplasia, parakeratosis, hyperkeratosis, and the formation of thickened adherent scale .Pathological changes of other organs were described.

Key words: *hypozincemiain, buffalo calves, parakeratosis, parakeratosis.*

Introduction

Buffalo is an economically valued livestock species in Egypt, One of the most important pillars of the Egyptian economy is livestock production. Among the most important sections of this production are the production of milk and meat. The great adaptive capacity of Egyptian water buffaloes (*Bubalusbubalis*) to tropical climates and excellent nutritional efficiency, resistance to diseases, together with the good productive and reproductive potential make these animals the main source for milk (about 60 % of total milk production) and meat (about 40% of total red meat production) in Egypt. However, this production is still not enough for our consumption (**Rizk, 2011**) . Zinc is an essential trace element required for the proper function of more than 100 enzymes and plays a crucial role in nucleic acid metabolism and essential for the survival and function of all cells. Deficiency then is expressed in a variety of disturbances of growth and repair (**Jones *et al*., 1997** and **Wilson *et al.*, 2006).** Several factors may affect the availability of zinc to ruminants and cause secondary zinc deficiency. These include the consumption of immature grass, feeding of late-cut hay and the presence of excessive dietary sulphur. .Moreover, the contamination of silage with soil at harvesting time can also affect the digestibility of zinc (**Radostits *et al.*, 2007**) Reduced appetite has been reported in buffalo calves affected with zinc deficiency (**Al-saad *et al*., 2006**). Zinc deficiency results in failure of keratinization, which leads to parakeratosis, loss and failure of growth of hair the lesions are most marked on the muzzle, vulva, anus, tail, head, ears, backs of the hind legs, knee folds, flank and neck.Lesions of coronary bands, retarded testicular development and ces-

sation of spermatogenesis. Most animals are below average body condition and are stuntedin growth .in addition to hypoplasia of the thymus, spleen and lymph node (**Jones *et al.*, 1997, Oberleas and Harland**, **2008**) . Hypozincemic calves may suffer from decreased growth rate, swelling of joints and stiff gait and pica. Paleness of mucus membranes, intermittent diarrhea, and loss of appétit were the most prominent symptoms of hypozincemia in cattle (**Al-saad *et al*., 2011)**. In hypozincemic sheep abnormal skin was common (hyperkeratosis, thickening, hardening and cracking) and alopecia was second most frequent sign (**Al-saad *et al*., 2010)**. Reduction in the mean values of RBc, HB and PCV in diseased cattle and buffaloes affected with hypozincemia, and anemia was of macrocyticnormochromic type. Also decrease in lymphocytes in diseasedbuffaloes cattle and calves affected with zinc deficiency (**Al-saad *et al.*, 2006** and **Al-saad *et al.*, 2011**). Decrease in the mean values of total platelets count, whereas significant increase was encountered in platelets volume. The serum level of AST and ALT were significantly higher in zinc deficient sheep (**Al-saad *et al*., 2010)**. Furthermore, copper, iron, selenium and manganese are involved in many biochemical processes supporting life (**Jones *et al.*, 1997)**.

The present study was planned after the signs of alopecia in different body regions and abnormal skin in buffalo calves herd to secure the following information:

Diagnostic procedures to determine the probable cause of skin abnormalities in buffalo calves as well as histopathological studies.

Detection of the mean levels of some biochemical constituents and hematological profile of blood in buffalo calves.

Detection of the mean levels of some minerals in diseased buffalo calves.

Materials and methods

Animals:

In a private farm in Marsamatroh (animal production unit) about thirteen buffalo animals out of fifty were suffering from loss of hair ,emaciation and abnormal skin. They were about 15-20 months old .Three of them was slaughtered for post mortem and histopathological examination. Bacteriological, virological and parasitological causes were excluded during examination of collected samples in different specific laboratories.

Samples:

1- Blood samples were obtained from (10 diseased and 5 healthy animals in another farm).Two blood samples were taken in clean and dry test tubes. One of them contains heparin as anticoagulant (1-2Iu|ml) for hematological examination. The other sample was left to clot and centrifuged for separation of clear serum and used for examination of different biochemical parameters. The heparinized samples were used for totalerythrocytic and leucocytic count using improved Neubouer haemocytometer (**Richard *et al*.1999**)

2- Ration samples: Two ration samples were taken from that given to diseased buffalo calves and apparently healthy calves . They were analyzed for determination of Ca, Ph, Mg copper, zinc, iron, crude protein and total fat as described by **AOAC (2010)**.

Pathological examination:

Postmortem examination was done immediately after slaughter and tissue specimens from skin, liver, spleen, kidney and lung were taken and fixed in 10%neutral buffered formalin, then processed for paraffin embedding section of 4-5 micron were prepared and stained with hematoxylin and eosin (H&E)(**Bancroft and Stevens 1996)**

Biochemical analysis:

Serum Aspartate aminotransferase (ALT) and Alanine aminotransferase(AST) **(Reitman and Frankel (1957),**Creatine kinase (CK) (**Szase and Busch 1979)** ,Lactate dehydrogenase LDH (**Caboud and Worblewski, 1958),** Urea (**Tabacco, 1979),** Creatinin (**Husdan and Rapoport, 1968),** Uric acid (**Fossati, *et al*., 1980)** Total Calcium (**Glinder and King 1972**) ,Total inorganic phosphorus (**Klichling and Freiburg 1951),** Magnesium (**Neill and**

Helly, 1956), sodium and potassium (**Oser, 1979**), serum selenium, zinc, manganese, iron and copper (**Burrieland Ramirez,1957).**

Statistical analysis:

Data obtained were statistically analyzed using t-student test according to **SPSS-14 (2006).**

Results and Discussion

Signs:

The main clinical signs, observed on the buffalo calves from a Marsamatroh farm were alopecia in different body regions, abnormal skin (rough, thickened, wrinkled, cracked with dandruff) paleness of mucous membrane, intermittent diarrhea, loss of appetite and decreased growth rate, the same results were reported in buffalo calves by **Al-Saad *et al.* (2006) .**It may attributed to the fact that the sense of taste is mediated through the salivary zinc dependent polypeptide, gustin. Low salivary zinc concentration leads to a reduction of taste and reduced appetite (**Droke *et al*., 1993**), swelling of joints and stiff gait and pica.Several previous studies showed similar clinical signs (**Al-Saad *et al*., 2010 and Al-Saad *et al.*,2011)**

Ration analysis:

Analysis of the collected ration samples revealed that both were balanced in their crude protein and total fat contents and free from rancidity. On the other hand zinc was low in the ration offered to the affected buffalo calves as presented in table (1).Deficient zinc in the presented ration was the main cause of the previous signs.

Hematological analysis:

Table (2) shows the hemogram of the apparently healthy and affected buffalo calves. The mean values of the RBCscount, Hb, PCV, MCV(mean corpuscular volume) and MCHC (mean corpuscular hemoglobin concentration) were significantly decreased in the affected buffalo calves when compared with the healthy one. These results pointed out coexistence of clinical marginal anemia with clinical laboratory mineral deficiency. Our results go hand in hand with (**Al-Saad *et al*.,2006, Al-Saad *et al*.,2010 and Al-Saad *et al.*,2011)**

The mean values of the total and differential leukocytic counts in the apparently healthy and diseased buffalo calves revealed non significant leucopenia in the diseased calves. Also increase eosinophils,basophils, monocytes and lymphocytes with decreased neutrophils were seen.The total leukocyticcount showednon significant decreased when compared with the healthy one. Such a decreased in the total leukocytic count may be attributed to anorexia and immunodepression (**Mogongo *et al.*,1985** and **Al-Saad *et al*., 2010)**.

Serum biochemical , some electrolyte and trace elements analysis:

The mean values of activities of serum AST, ALT, CK and LDH in the healthy and affected buffalo calves are presented in Table (3). They were significantly increased in the affected buffalo calves. Hepatic damage was monitored by the significant increase in the AST, ALT, CK and LDH activities in the diseased animals. Elevated liver enzymes in the blood of buffalo calves associated with zinc hypozincemia indicated to hepatic dysfunction(**Al-Saad*et al*.,2010)**.

Results in Table(3) also indicated a significant elevated of serum urea, uric acid and creatinine in the affected animals, similar findings were previously observe**(Ali, 1992** and **Meschy, 2000)**.

The significant decrease in the serum calcium, phosphorus, magnesium, sodium and potassium levelsTable (4) in the diseased buffalo calves could be attributed to presence of diarrhea which decreased the intestinal absorption (**Abdel-All *et al*., 1990)**. Copper, zinc, iron, selenium and manganese were decreased in the diseased animals may be attributed to interference with their absorption from the gastrointestinal tract beside anorexia. Decrease of these minerals explained the changes of hematological profile of diseased buffalo calves.

Table (1). Analysis of ration offered to diseased and apparently healthy animals

Parameter	Ration of feed to diseased animals	Ration of feed to apparently healthy animals
Crude Protein%	15.8	15.6
Total Fat%	3.2	3.5
Ca%	0.93	0.89
Ph%	0.48	0.51
Mg%	0.32	0.30
Cu%	0.4	0.38
Iron %	4.3	4.2
Zinc%	0.052	0.25
Acid No.	6.4	7.3

Table (2). Hematological finding in affected and apparently healthy buffaloes (Mean±SE).

Hematological parameter		Apparently healthy n=5	Affected animals n=10
RBCs(10^6/ul)		9.82±0.46	6.64±0.45**
Hb(gm/dl)		12.82±0.49	9.04±0.37**
PVC(%)		38.64±1.02	31.14±0.62**
MCV(F1)		44.03±1.99	48.0±2.17**
MCH(Pg/cell)		13.22±0.53	13.48±1.01
MCHC(gm/dl)		33.61±1.13	28.35±1.07**
TLC(10^3/ul)		9.5±0.37	9.0±0.14
Absolute differential leucocytic count (10^3/ul)	E	0.45±0.07	0.52±0.04
	B	0.408±0.0002	0.52±0.04
	M	0.60±0.01	0.64±0.025
	L	4.20±0.18	4.45±0.25
	N	3.85±0.06	2.87±0.2

Table (3). Serum biochemical parameters of the affected and apparently healthy animals (Mean±SE)

Parameter	Apparently healthy n=5	Affected animals n=10
ALT(u/l)	85.5±2.1	117.3±3.2**
AST(u/l)	15.3±0.32	77.3±1.2**
CK(Iu/l)	170±6.25	390±18.4**
LDH(Iu/l)	1250±90.1	2670±80.4**
Urea(mg/dl)	38.4±1.2	49±1.4**
Creatinin(mg/dl)	0.92±0.04	1.84±0.06**
Uric acid(mg/dl)	7.1±0.35	8.4±0.44*

Table (4). Serum analysis of some electrolytes and trace element in affected and apparently healthy buffaloes (Mean±SE)

Parameter	Apparently healthy n=5	Affected animals n=10
Total calcium(mg/dl)	2.75±0.06	1.92±0.03*
Total phosphorus(mg/dl)	1.65±0.07	1.02±0.04*
Total magnesium(mg/dl)	1.16±0.1	0.92±0.1*
Sodium(mmol/L)	141±2.1	130±1.98*
Potassium(mmol/L)	3.1±0.31	5.1±0.4**
Manganese(µg/dl)	68.2±3.1	39±4.2**
Zinc(µg/dl)	80.08±6.1	35±4.1***
Selenium(mg/dl)	8.28±0.45	6.18±0.09*
Iron(µg/ld)	87.3±4.1	63±1.3*
Copper(µg/ld)	60.3±2.6	49.8±3.1**

n= Number of buffalo calves

*=Significant **=Highly Significant ***=Very Highly Significant (P≤0.001)

Pathological examination:

Macroscopically

Skin of affected buffalo calves showed alopecia in different body regions, abnormal skin (rough, thickened, wrinkled, cracked with dandruff). Keratinization and failure of growth of hair. The lesions are most marked on the muzzle, vulva, anus, tail, head, ears, backs of the hind legs, knee folds, flank and neck. The results are similar to those reported by (**Al-Saad *et al*., 2006**).

The liver of some diseased buffalo calves were enlarged, soft, pale and its capsule showed petechial hemorrhage. These changes were previously cited by (**Gehan *et al*., 2011**). Meanwhile the kidneys were enlarged and petechial hemorrhages were prominent which agreed with findings of (**Gehan *et al*., 2011**). The spleen was atrophied and sometimes hemorrhagic spots were present.

Microscopically:

Skin of buffalo calves affected with hypozincemia showing increased thickness of the epidermis, with or thokeratotic, parakeratotic and hyperkeratosis,(fig 1,2). Some bacteria invaded broken skin wereseen as basophilic bacterial colonies (fig 3), vesicopustule containing pale eosinophilic material with numerous neutrophils were found (fig 4). Thickened adherent scale in some partsandfocal ulceration with leukocytic infiltration. The results are due to zinc deficiency where zinc is an integral component of a wide range of metalloenzymes and acts as a cofactor for RNA and DNA polymerases (**Mozaffari and Derakhshanfar, 2007**), and its presence is of particular importance in rapidly-dividing cells, including those of the epidermis (**Nishi, 1996**). Zinc is also essential for the biosynthesis of fatty acids and participates in both the inflammatory and immune responses and also involved in the metabolism of vitamin A (**Watson, 1998**). Liver of buffalo calves affected with hypozincemia shows portal area with biliary hyperplasia, congested blood vessels and round cells infiltration (fig 5), besides vacuolar degeneration in hepatocytes (fig 6). The lesions which were reported may be due to zinc deficiencywhich plays a crucial role in nucleic acid metabolism and essential for the survival and function of all cells. So deficiency causes variety of disturbances in repair (**Jones *et al*., 1997)**, also (**Godson *et al*. 1995)**suggested that the dilatation and leakage of blood vessels, accumulation of leukocytes and activation of stromal cells of liver to release biological response inhypozincemic cases. Kidneys of affected calves revealed necrotic renal tubules (tubulonecrosis), focal interstitial aggregation of round cells (fig 7) and cystic dilation of some renal tubules and shrunken glomerulous (fig,8). The obtained results of microscopic examination of liver and kidney revealed the significant elevation of level of liver enzymes (AST and ALT) and the level of kidney functions (urea and Creatinine) which were affected with zinc deficiency. Spleen of buffalo affected with hypozincemia showing depletion of lymphocytes in the white pulp and thickening of spleenic fibromuscular capsule (fig 9, 10).Lung of buffalo calves affected with hypozincemia showed edema and hypertrophied mesothelial cells of pleura (fig 11) and bronchitiswith desquamated lining epithelia with round cells infiltrations (fig 12). The histopathological changes reported in buffalo calves were nearly agreed to those reported in zinc deficiency in animal swith (**Jones *et al*.,1997; Singer *et al*., 2000; Tyler *et al*.,2003Al-Saad *et al*.,2006; Al-Saad *et al*., 2010** and **Al-Saad *et al.*, 2011)**. These changes are commonly reported in zinc deficiency but are not entirely diagnostic for this type of deficiency. They have been attributed to the involvement of zinc, copper and selenium in many biochemical processing supporting life such as utilization of oxygen, DNA and RNA reproduction, maintenance of cell membrane integrity and sequestration of free radicals (**Wright and Spears, 2004**).

Conclusion:

From the present study it could be concluded that:

1- Deficient of zinc was the main cause of the signs (skin abnormalities, intermittent diarrhea and loss of appetite) in the buffalo calves.

2- Copper, iron, selenium and manganese as

well as zinc which were decreased in the diseased animalsexplainedthe changes of hematological profile and serum biochemical analysis of diseased calves.

3- Pathological changes of skin, liver, kidneys ,spleen and lungs of affected buffalo calves were related to zinc and other minerals deficiency.

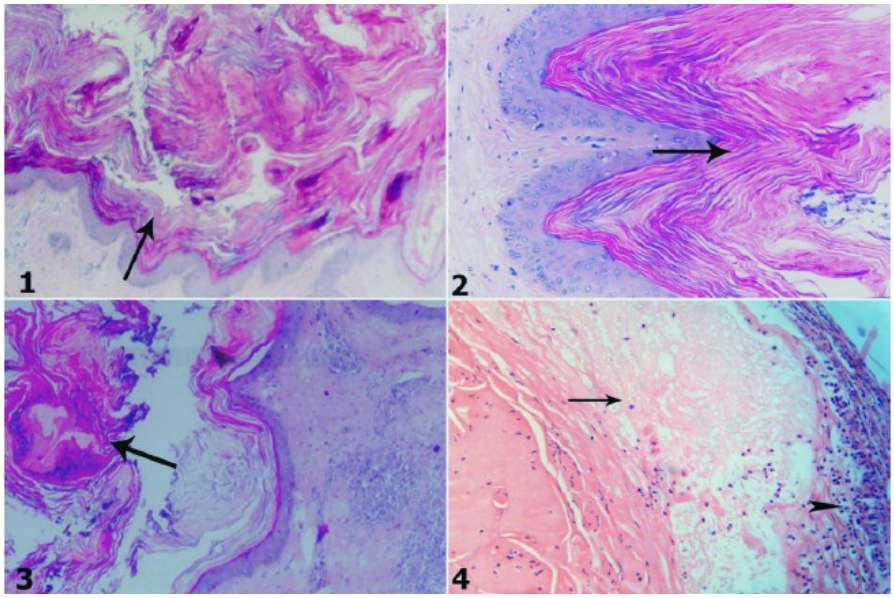


Fig "1,2": Skin of buffalo calf shows increased thickness of the epidermis, with orthokeratotic,parakeratoticand hyperkeratosis (H&E)(1×400&2×200).
Fig" 3": Some basophilic bacterial colonies (H&E×200).
Fig "4":vesicopustule containing pale eosinophlic material with numerous neutrophils are found(H&E.×200).

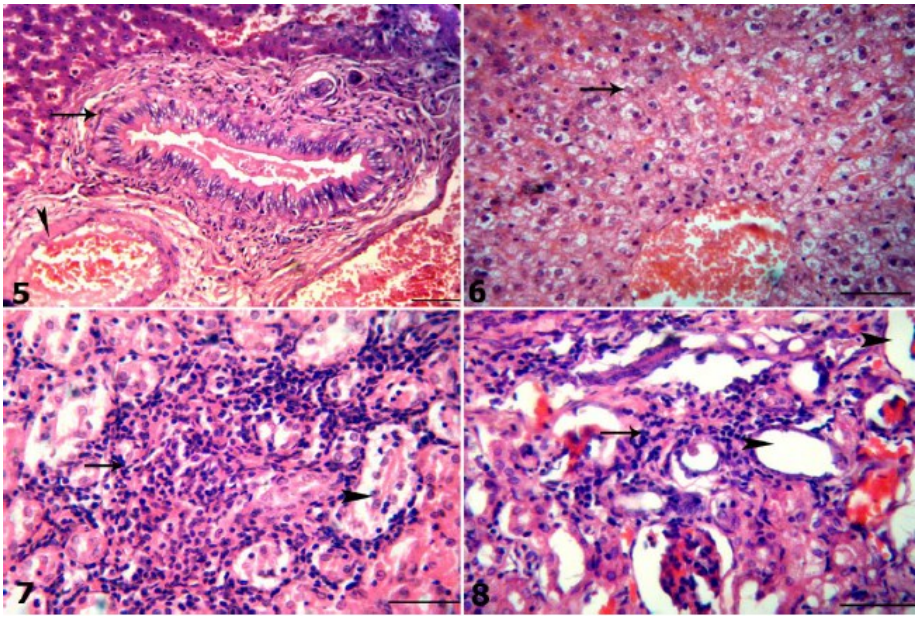
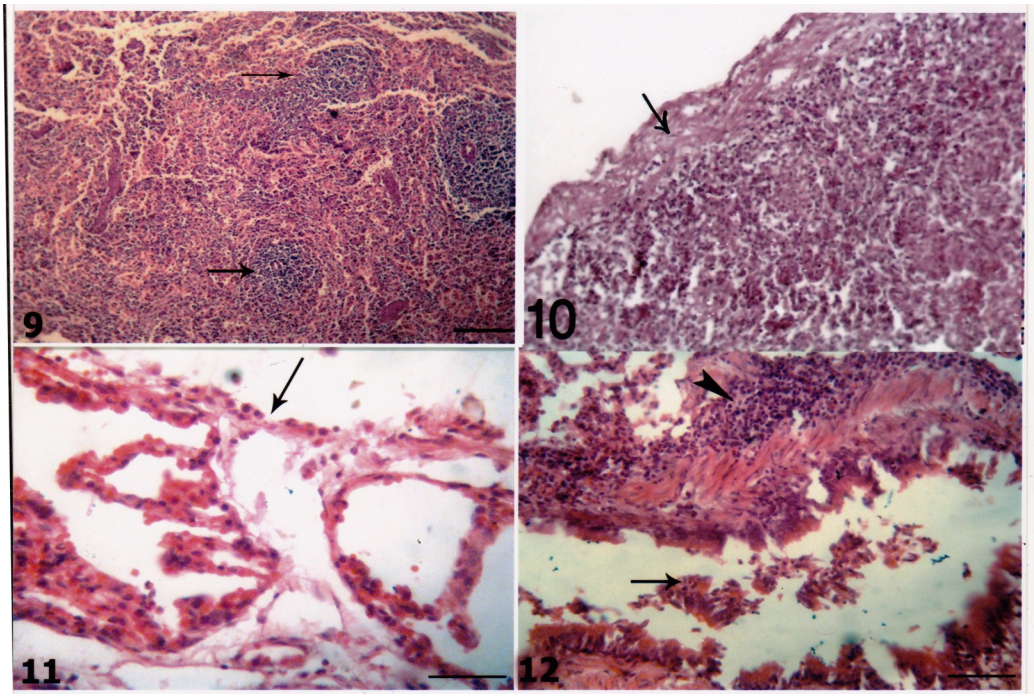


Fig "5": Liver of buffalo calf shows portal area with biliary hyperplasia, congested blood vessels with few round cells infiltration.(H&E×200)
Fig"6": Liver shows vacuolar degeneration in hepatocytes.(H&E×200)
Fig"7": Kidneys of affected calf shows tubulonecrosis with focal interstitial aggregation of round cells (H&E×400)
Fig"8": cystic dilation of some renal tubules and shrunken glomerulous.(H&E×400)



Fig"9": Spleen of buffalo calf showsmoderate depletion of lymphocytes in the whitepulp (H&E×120)
Fig"10": Spleen of buffalo calf shows thickening of splenic fibromuscular capsule.(H&E×120)
Fig"11": Lung of buffalo calf shows edema of pleura (H&E×200)
Fig"12": Lung of buffalo calf showsbronchitis with desquamated lining epithelia with round cells infiltrations, (H&E×200)

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دراسات هيماتولوجية وبيوكيمائية و باثولوجية لنقص المعادن مع إشارة خاصة لنقص الزنك فى الجاموس المصرى

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الملخص العربى

تمت دراسة التغيرات الدموية والمرضية وبعض قياسات الكيمياء الحيوية لعجول جاموس مصابة طبيعيا بنقص الزنك فى مزرعة خاصه بسخا . تم فحص 10 عجول يتراوح عمرها بين 15 و 20 شهر كذلك فحصت 5 عجول سليمة ظاهريا" و فى نفس العمر و استخدمت كضابط للدراسة .

عجول الجاموس المصابة اظهرت فقدان للشعر فى أماكن مختلفة من الجسم , جلد غير طبيعى (خشن , سميك , مجعد و به تشققات وقشور) . شحوب بالأغشية المخاطية , اسهال متقطع , فقدان للشهية مع الميل لأكل أشياء غريبة أدى إلى نقصان الوزن . تورم المفاصل مع تعسر بالحركة .

التحليل الاحصائى أظهر نقص معنوى بعدد كرات الدم الحمراء و الهيموجلوبين .

نتائج الكيمياء الحيوية أظهرت نقص معنوى فى الزنك وبعض الأملاح المعدنية الأخرى (الماغنسيوم , النحاس , الحديد), كذلك زيادة فى إنزيمات الكبد و اليوريا و الكرياتينين فى العجول المصابة بنقص الزنك.

تم تسجيل النتائج بالفحص المجهرى للجلد المصاب والأعضاء الدلخلية الأخرى للعجول.