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# Effect of ferrous sulphate on haematological, biochemical and immunological parameters in neonatal calves

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**Summary**

The effect of oral administration of iron on haematological, biochemical and immunological parameters in neonatal calves was studied. Ten calves from a private farm in Gharbia Governorate were used. Calves were separated from their dams immediately after birth and received colostrum during the first hours after calving and twice daily for 48 h. Thereafter, they received whole milk. Calves were divided into two equal groups. The first group was kept as controls. Calves of the second group were given ferrous sulphate at a dose of 250 mg/calf daily, beginning at one day of age; this was continued for 28 days. Three blood samples were collected from each calf in all groups at 14, 21, 28 and 35 days of age. Iron administration produced a significant increase in red blood cell count, haemoglobin, packed cell volume and blood indices, in addition to non-significant changes in total and differential leukocyte counts. The administration of iron resulted in a significant increase in serum iron, total proteins, globulins, thyroid hormones, lymphocyte stimulation index, phagocytosis, body weight and body gain. The administration of iron is suggested as routine practice in calf-producing farms due to its advantageous effects on the parameters tested.

**Keywords**

Biochemistry, Calf, Egypt, Haematology, Immunity, Iron, Nutrition.

## Effetto del solfato di ferro su parametri ematologici, biochimici e immunologici in vitelli neonati

**Riassunto**

*E' stato studiato l'effetto della somministrazione orale di solfato di ferro su parametri ematologici, biochimici e immunologici in vitelli neonati. Sono stati utilizzati dieci vitelli provenienti da un'azienda privata nel Governatorato di Gharbia. I vitelli sono stati separati dalle madri subito dopo la nascita, è stato somministrato loro il colostro nelle prime ore dopo il parto e due volte al giorno per 48 ore. In seguito, è stato somministrato latte intero. I vitelli sono stati divisi in due gruppi numericamente uguali, il primo impiegato come gruppo di controllo, il secondo soggetto alla somministrazione di solfato di ferro alla dose di 250 mg al giorno per animale, a partire dal primo giorno di vita e per 28 giorni. Sono stati raccolti tre campioni di sangue da ogni vitello dei due gruppi a 14, 21, 28 e 35 giorni di età. La somministrazione del solfato di ferro ha prodotto un significativo incremento di globuli rossi, emoglobina, valore ematocrito e altri indici ematici, oltre a modifiche non significative della conta totale e differenziale dei leucociti. La somministrazione di solfato di ferro ha determinato un significativo aumento della sideremia, delle proteine totali, delle globuline, degli ormoni tiroidei, dell'indice di stimolazione linfocitaria, della fagocitosi e del peso corporeo. La somministrazione di solfato di ferro è consigliata come pratica di*

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*routine nelle aziende produttrici di vitelli per i suoi effetti vantaggiosi in favore dei parametri testati.*

**Parole chiave**
Biochimica, Egitto, Ematologia, Immunità, Nutrizione, Solfato di ferro, Vitello.

# Introduction

Iron is a nutrient that is related to health and immunity (16). It is the second most common element on Earth. Unfortunately, iron is chemically unstable and is easily oxidised into an insoluble ferric form. Ferric iron is unavailable in most biological systems. All living organisms, with the exception of lactobacillus, require iron for their metabolism (24). Iron is an essential component of haemoglobin, myoglobin and several enzymes, such as catalase, peroxidase and cytochrome oxidase (15). The requirements in iron of ruminants are not well established and most recommendations are estimates (28).

It is generally accepted that the iron requirements of young animals are greater than those of mature ruminants and are thought to be 100 ppm. Deficiencies are most likely to occur in young animals because cow's milk is low in iron (about 10 ppm). The iron reserves of the calf, which are primarily in the liver, are generally sufficient to prevent serious anaemia. If calves are fed an exclusively milk diet for several weeks, they may develop iron deficiency anaemia which can adversely affect growth and feed conversion (25). Iron deficiency anaemia is a frequent disorder in calves. Iron deficiency is associated with numerous clinical signs, including anaemia, reduced growth and increased disease rates. Cell mediated immunity, the number of neutrophils with phagocytic activity, activity of iron containing enzymes and serum IgG were significantly reduced in iron-deficient calves (14). However, numerous studies concluded that oral or parenteral administration of iron provided an increase in haematological parameters and better performance in calves (6, 14, 16, 23). Therefore, this study was designed to clarify the effect of oral administration of ferrous sulphate on haematological, biochemical and immunological parameters in newborn calves.

# Materials and methods

## Experimental calves

This study was conducted on a private farm in the Gharbia Governorate. Ten calves were used. Calves were separated from their dams immediately after birth and held in boxes with straw. All calves were fed colostrum during the first hours of life (2 kg/calf) then twice daily for the next 48 h. Thereafter, calves were fed mature milk (5% of body weight) for the entire experimental period.

## Experimental design

Ten calves of both sexes were divided into two equal groups. The first group was kept as controls. Calves in the second group received ferrous sulphate orally at a dose of 250 mg/calf/day from one to 28 days old.

## Blood sampling

The first administration of ferrous sulphate began at one day of age. Blood samples were collected at 14, 21, 28 and 35 days of age. Three blood samples were collected from each calf in all groups at 14, 21, 28 and 35 days of age. The first blood sample was collected in a test tube containing ethylene-diamine-tetraacetic acid (EDTA) and used for haematological examinations. The second blood sample was collected in centrifuge tubes, left to clot and centrifuged for separation of serum and used for biochemical analysis. The third blood sample was collected in heparinised syringes in aseptic conditions and used for cellular immune function tests.

## Haematological examinations

Red blood cell (RBC) counts, packed cell volume (PCV) and total and differential leukocytic counts were performed (9). Haemoglobin was measured colourimetrically (11).

## Serum biochemical assay

Serum iron was measured using a Boehringer-Mannheim kit with a Hitachi colourimeter. Total serum proteins and albumin were determined colourimetrically using commercial

kits (BioMérieux, Marcy l'Etoile). Serum globulin was determined by subtracting the serum albumin obtained from total serum proteins (10). Thyroid hormones were measured by radio immunoassay (1).

### Cellular immune function tests

A lymphocyte transformation test was performed (26) and phagocytosis was measured (30).

### Performance

Body weight and weight gain were recorded at the end of the experimental period.

### Statistical analysis

All data obtained were analysed using Student's t-test (29).

## Results and discussion

Iron is an essential component of a number of proteins involved in oxygen transport and utilisation. These proteins include haemoglobin, myoglobin and a number of cytochromes. Several enzymes also either contain iron or are activated by iron. Iron has been identified as a necessary element for the normal development of newborn calves and, to the contrary, milk is considered a poor source of iron (3). In our study, iron administration elicited a significant increase in RBC count, haemoglobin concentration, PCV and mean corpuscular haemoglobin concentration (MCHC) when compared with non-supplemented control calves at 14, 21, 28 and 35 day of age (Table I). Calves in the control group suffered from microcytic hypochromic anaemia. An adequate iron supply is necessary for production of RBCs and haemoglobins. A progressive reduction in RBCs, haemoglobin and PCV occur during the first weeks of life (16, 22). Moreover, the most critical period of anaemia in calves was the first 3-5 weeks of age (5). RBC parameters are used most commonly to monitor erythropiosis. Sufficient erythropiosis was reached with a sufficient iron supply. Most iron, whether administered orally or parenterally, was utilised by RBCs and only a small amount was assimilated at storage sites. Neonatal calves responded well to the administration of iron (21). In this study, the increase in RBC parameters was seen to be concomitant with iron administration. Various studies recorded that iron administration provided an increase in RBC parameters in calves (6, 16, 20, 22, 27). Oral administration of iron produced non-significant changes in total and differential leukocyte counts when compared with control calves (Table II).

Table I
Erythrogram in neonatal calves that had received iron, in comparison to the controls (mean values ± SE)

| Age (days) | RBCs($10^6$/µl) | | Haemoglobin (gm/dl) | | PCV (%) | | MCV (fl) | | MCH (Pg) | | MCHC (%) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C | T | C | T | C | T | C | T | C | T | C | T |
| 14 | 8.28 ± 0.46 | 8.86 ± 0.31 | 10.1 ± 0.36 | 12.01 ± 0.16* | 29 ± 0.12 | 34 ± 0.11* | 35.02 ± 1.19 | 38.37 ± 1.12* | 12.19 ± 0.26 | 13.55 ± 0.22 | 34.80 ± 1.01 | 36.62 ± 1.16* |
| 21 | 7.88 ± 0.16 | 9.51 ± 0.22* | 9.12 ± 0.14 | 11.98 ± 0.13* | 27 ± 0.16 | 35 ± 0.26** | 34.26 ± 1.29 | 37.85 ± 1.82* | 11.57 ± 0.29 | 12.59 ± 0.19 | 33.67 ± 1.21 | 36.17 ± 1.62* |
| 28 | 7.82 ± 0.31 | 9.91 ± 0.62* | 8.76 ± 0.36 | 12.56 ± 0.64* | 28 ± 0.18 | 38 ± 0.16** | 35.80 ± 2.12 | 38.34 ± 2.26* | 11.20 ± 0.31 | 12.67 ± 0.19 | 31.82 ± 1.34 | 35.05 ± 1.49* |
| 35 | 7.80 ± 0.63 | 10.23 ± 0.42* | 8.82 ± 0.13 | 12.41 ± 0.63* | 28 ± 0.18 | 39 ± 0.26** | 35.89 ± 1.23 | 38.12 ± 1.14* | 11.31 ± 0.25 | 12.13 ± 0.29 | 31.50 ± 1.02 | 35.09 ± 1.36* |

* significant at $p<0.05$ (a mean value in a row beneath the same heading and followed by an asterisk is significantly different at $p<0.05$)

** significant at $p<0.01$ (a mean value in a row beneath the same heading and followed by two asterisks is significantly different at $p<0.01$)

C control
T test
RBCs red blood cells
PCV packed cell volume
MCH mean corpuscular haemoglobin
MCHC mean corpuscular haemoglobin concentration

Table II
Leukogram in neonatal calves that had received iron, in comparison to controls (mean values ± SE)

| Age (age) | TLC ($10^3$/µl) | | Neutrophils (%) | | Lymphocytes (%) | | Monocytes (%) | | Eosinophils (%) | | Basophils (%) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C | T | C | T | C | T | C | T | C | T | C | T |
| 14 | 11.60 ± 0.28 | 12.03 ± 0.62 | 31.14 ± 1.1 | 31.11 ± 1.9 | 58.41 ± 1.2 | 60.31 ± 1.3 | 3.52 ± 0.3 | 3.56 ± 0.6 | 2.82 ± 0.1 | 2.81 ± 0.2 | 2.11 ± 0.01 | 2.22 ± 0.03 |
| 21 | 10.63 ± 0.29 | 11.18 ± 0.62 | 31.91 ± 1.6 | 32.12 ± 1.2 | 56.32 ± 1.6 | 61.20 ± 1.9 | 3.48 ± 0.2 | 3.03 ± 0.3 | 2.80 ± 0.1 | 2.69 ± 0.2 | 2.28 ± 0.06 | 1.96 ± 0.06 |
| 28 | 10.28 ± 0.13 | 11.96 ± 0.18 | 32.23 ± 2.2 | 31.10 ± 1.8 | 58.11 ± 1.9 | 60.12 ± 1.3 | 3.20 ± 0.2 | 3.51 ± 0.1 | 2.81 ± 0.1 | 2.82 ± 0.3 | 2.25 ± 0.03 | 2.67 ± 0.05 |
| 35 | 10.56 ± 0.25 | 11.05 ± 0.22 | 32.12 ± 1.82 | 32.19 ± 1.5 | 58.10 ± 1.3 | 60.21 ± 1.6 | 3.50 ± 0.3 | 3.14 ± 0.2 | 2.81 ± 0.2 | 2.36 ± 0.29 | 2.47 ± 0.08 | 2.19 ± 0.01 |

TLC total leukocytic count
C control
T test

In our study, the oral administration of iron elicited a significant increase in serum iron at 14, 21, 28 and 35 days of age (Table III). Our results agreed with those of others (5, 6, 8, 22, 23, 27). The iron requirements of domestic animals are influenced by age, growth rate and availability of iron source. Iron is generally supplemented as ferrous sulphate, ferrous carbonate or ferrous oxide. The availability of iron is highest for ferrous sulphate (24). Our results regarding serum iron were in accordance with results reported previously (16, 22, 23).

In our study, iron administration lead to a significant increase in total serum proteins and globulin at days 21, 28 and 35, in addition to non-significant changes in serum albumin (Table III). Hyperglobulinaemia in iron-supplemented calves may be due to the role of iron in immunity. Iron is a nutrient related to health and immunity (7). The immune system is an extremely complex system that can be affected by many factors including nutrition. Trace minerals (iron, zinc and selenium) play a critical role in the development and maintenance of the immune system. While required in very small quantities, trace minerals function as a critical component of various enzyme systems in the control of cellular activities that produce the specific immunoglobulins required for immune response in the animal (2, 12). Similar results

Table III
Serum biochemical parameters in in neonatal calves that had received iron, in comparison to controls (mean values ± SE)

| Age (days) | Serum iron (µmol/l) | | Total proteins (g/dl) | | Albumin (g/dl) | | Globulin (g/dl) | | T3 (ng/ml) | | T4 (ng/ml) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C | T | C | T | C | T | C | T | C | T | C | T |
| 14 | 16.94 ± 1.73 | 19.46 ± 1.92* | 4.92 ± 0.31 | 5.12 ± 0.62* | 2.69 ± 0.11 | 2.65 ± 0.08 | 2.24 ± 0.09 | 2.47 ± 0.03 | 3.60 ± 0.3 | 3.85 ± 0.1 | 86 ± 2.2 | 92 ± 3.5* |
| 21 | 14.99 ± 1.02 | 22.03 ± 1.31* | 5.01 ± 0.16 | 5.86 ± 0.13* | 2.72 ± 0.13 | 2.74 ± 0.16 | 2.29 ± 0.10 | 3.12 ± 0.06* | 3.51 ± 0.1 | 4.12 ± 0.5* | 82 ± 5.8 | 106 ± 6.4* |
| 28 | 16.02 ± 1.31 | 25.38 ± 2.18** | 4.98 ± 0.11 | 5.92 ± 0.13* | 2.66 ± 0.11 | 2.69 ± 0.09 | 2.32 ± 0.06 | 3.24 ± 0.09* | 3.28 ± 0.2 | 4.69 ± 0.1* | 83 ± 4.1 | 112 ± 3.8* |
| 35 | 16.72 ± 0.36 | 28.76 ± 1.68** | 4.91 ± 0.13 | 5.98 ± 0.03* | 2.70 ± 0.15 | 2.66 ± 0.16 | 2.12 ± 0.07 | 3.18 ± 0.08* | 3.18 ± 0.2 | 4.99 ± 0.3* | 79 ± 3.2 | 115 ± 4.6* |

* significant at $p<0.05$ (a mean value in a row beneath the same heading and followed by an asterisk is significantly different at $p<0.05$)
** significant at $p<0.01$ (a mean value in a row beneath the same heading and followed by two asterisks is significantly different at $p<0.01$)
C control
T test

have been published (14) proving that serum immunoglobulin concentrations increase in iron-supplemented calves.

Oral administration of iron to newborn calves produced a significant increase in triiodothyronine and thyroxin when compared with those of the control calves (Table III). Studies in animals have shown that iron deficiency anaemia (IDA) impairs the thyroid metabolism. The IDA decreases the serum thyroid hormone concentration. The mechanism by which iron influences thyroid and iodine metabolism is still unclear. The IDA could impair the thyroid metabolism through anaemia and reduced oxygen transport. The IDA may also alter the central nervous system control of the thyroid metabolism and nuclear T3 binding (4). Another potential mechanism is impairment of thyroid peroxidase activity (TPO). The TPO is an iron-dependent enzyme located at the apical membrane of the thyrocyte. The TPO catalyses the first two steps of thyroid hormone synthesis, namely: iodination of thyroglobulin and coupling of iodotyrosine residues (13). The IDA lowers the activities of other heme containing enzymes, i.e. cytochrome oxidase, myeloperoxidase and succinate-oxidoreductase. Similar results have been reported previously (4, 17, 28). In regard to the effect of oral administration of iron on the cellular immune function, it produced a significant increase in the lymphocyte stimulation index and phagocytosis percent (Table IV). Cell mediated immunity, number of neutrophils with phagocytic capacity and activity of iron containing enzymes, myeloperoxidase and serum IgG concentration significantly increased in iron-supplemented calves (14). T-lymphocytes are the primary group which the body relies on for cell mediated immunity which activates macrophages, natural killer cells and the release of cytokines. The original T-cells are produced in the thymus without being targeted for specific disease challenge. When T-cells are exposed to an antigen, metabolic changes occur in the T-cells, which leave them sensitised to the specific antigen. These metabolically changed T-cells are the memory cells which remain active in the body for long periods. If a memory cell is re-exposed to the same antigen, T-lymphocyte, macrophages and cytokines will be targeted towards the cells containing that antigen. T-cells and other cells of the immune system destroy the cell containing that antigen. Normal development and the function of T-cells depend on an adequate supply of iron, zinc and copper. Thymuline is a hormone involved in T-lymphocyte maturation which is known to be zinc-dependent. Copper deficiency inhibits the formation of interleukin which regulates the T-cell function, while iron deficiency can inhibit the maturation process of T-cells. Trace minerals (iron, zinc and copper) are not only important for the formation and function of lymphocytes but also for the acceleration of the speed at which lymphocytes can react to a disease challenge (2). Concerning the effect of iron administration on the performance of calves, it produced a significant increase in total body weight and body gain at 35 days of age when compared to non-supplemented control calves (Table V). A correlation between iron and RBC parameters with performance has been clarified previously (14, 19, 22, 23). In calves with an iron deficiency, insulin-like growth factors (IG-I) and its response to

Table IV
Cellular immunity tests
(mean values ± SE) in neonatal calves (test) that had received iron, in comparison to controls

| Age (days) | Lymphocyte stimulation index | | Phagocytosis (%) | |
|---|---|---|---|---|
| | Control | Test | Control | Test |
| 14 | 1.26 ± 0.12 | 1.59 ± 0.11* | 76.81 ± 1.13 | 87.12 ± 1.59* |
| 21 | 1.32 ± 0.09 | 1.82 ± 0.02* | 75.68 ± 3.16 | 89.32 ± 2.82* |
| 28 | 1.29 ± 0.08 | 1.86 ± 0.13* | 78.12 ± 2.16 | 88.31 ± 2.16* |
| 35 | 1.25 ± 0.01 | 1.78 ± 0.02* | 77.28 ± 2.36 | 87.22 ± 2.44* |

* significant at $p<0.05$ (a mean value in a row beneath the same heading and followed by an asterisk is significantly different at $p<0.05$)

Table V
Performance of in neonatal calves (test) that had received iron, in comparison to controls (mean values ± SE)

| Parameter | Control | Test |
|---|---|---|
| Total weight gain /kg | 7.22 ± 0.21 | 13.68 ± 0.69* |
| Total body weight/kg | 35.19 ± 1.92 | 49.26 ± 1.31* |

* significant at $p<0.01$ (a mean value in a row beneath the same heading and followed by an asterisk is significantly different at $p<0.01$)

exogenous somatotrophin are reduced. Food intake, average daily weight gain and growth were reduced in iron-deficient calves (18). It appears that sufficient iron is required for a normal appetite, secretion of IG-I and triiodothyronin and glucose utilisation.

## Conclusion

Our results indicate that the administration of iron should be established as accepted practice in calf-producing farms due to its advantageous effects on RBC parameters, serum iron concentrations, serum globulin, thyroid hormones, cellular immune functions and performance.

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