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FERROKINETIC STUDIES IN NORMAL AND IRON DEFICIENCY ANEMIC CALVES

By

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MÖLLERBERG, LARS, LARS EKMAN and STEN-OLOF JACOBSSON: *Ferrokinetic studies in normal and iron deficiency anemic calves*. Acta vet. scand. 1975, 16, 205—217. — Ferrokinetic studies were performed on control calves and on calves with experimentally induced iron deficiency anemia, all 15 weeks old.

The plasma iron clearance half time was about 4 times shorter in the experimental than in the control group. The low plasma iron concentration in the anemic calves was partially compensated by a more rapid plasma iron disappearance. Therefore the difference in the plasma iron turnover rate was reduced.

The mean value of plasma iron daily renewal rate was about 3 times higher in the experimental than in the control group.

The maximum uptake of injected ^{59}Fe into blood cells was reached 14 to 16 days after injection. The uptake of ^{59}Fe was about 10 % higher in the control than in the experimental group.

Using the values from the ferrokinetic study, the iron need for calves could be estimated. The requirement of iron to maintain a normal and constant Hb in a calf weighing 100 kg at a growth rate of 1 kg/daily was estimated as being 17.5 mg/day. Based on information in the literature and assuming a retention of dietary Fe of 25 %, the total daily iron need for such a calf gaining 1 kg/day would be 160—180 mg.

ferrokinetics; calves; iron deficiency anemia.

In the intensive systems of calf rearing there is still a risk of deficiency diseases e.g. iron deficiency anemia (Matzen 1968, Scheidegger 1973 and Möllerberg *et al.* 1975 b). It has been shown that iron supplementation of iron deficiency anemic calves resulted in higher growth rate and a lower incidence of illness. However, the iron supplementation must be appropriate, because the susceptibility to bacterial and fungal pathogens has been reported to be greater during hyperferremic episodes than in periods when the plasma iron is within the normal range (Weinberg 1974). Also as iron intoxications have been reported in other species, this possibility must be taken into consideration

(Tollerz 1965). Ferrokinetic studies in normal and anemic calves can give basic information on the estimation of adequate iron supplementation to intensively reared calves. Only limited investigations dealing mainly with healthy calves, have previously been reported (Baker & Douglas 1957, Hansard et al. 1959, Kaneko & Mattheeuws 1966).

MATERIAL AND METHODS

Eight calves of the Swedish Red and White breed born within 8 days were used in the experiment. The calves were fed milk (2×2 l daily) until an average age of 10 days. The calves were then divided on the basis of age, weight and hemoglobin values into 1 experimental and 1 control group with 4 animals in each (Table 1). Each experimental calf was put alone in a wooden box and fed only milk substitute ("Kalvex", the Swedish Farmers' Purchasing Association, Stockholm) according to the requirements for veal calves. The milk substitute contained 19 mg Fe/kg. During the experimental period from an age of about 10 days to 18 weeks the daily ration increased successively from 680 to 2270 g of milk substitute. The control calves were put in boxes with straw beds. These calves received the equivalent of 4 l of milk per day in the form of milk substitute, hay ad libitum and 0.1 to 0.5 kg concentrate during the first 8 weeks. After that the calves were

Table 1. Status of calves as regards age, weight, and Hb values at the beginning of the experiment.

Calf no.*	Age (days)	Weight (kg)	Hb (g/100 ml)
12-C	9	40	11.3
13-C	9	38	13.6
52-C	15	41	12.3
54-C	8	38	10.4
11-E	9	39	11.1
14-E	9	41	11.8
15-E	7	36	10.6
16-E	13	45	14.5
Mean for C	10.3	39.3	11.9
Mean for E	9.5	40.3	12.0

* C = Control calves to be kept on normal diet throughout the experiment.

E = Experimental calves to be kept on iron deficiency diet.

fed hay, water and later on concentrate ad libitum. The calves were clinically examined daily. If severe diarrhea was noticed, the amount of milk replacer was reduced until the feces had a normal consistency. If this had no effect the animals were treated with antibiotics for 5 days.

The calves were weighed at the beginning of the experiment, directly after ^{59}Fe was injected and then every 4th day.

Blood samples for the hematological examinations were taken the day before the calves were divided into 2 groups and then at intervals as indicated in Figs. 1—2 until the ferrokinetic study began. Thereafter the packed cell volume (PCV) and hemoglobin (Hb) were determined every second day until the end of the study. The methods used for the determinations of Hb, PCV, erythrocytes (R.C.), serum iron (SI) and unsaturated iron binding capacity (UIBC) were the same as described elsewhere (Möllerberg 1975).

The ferrokinetic study began 14 weeks after dividing the calves into 2 groups. The calves were given i.v. 50—100 μCi ^{59}Fe as ferric chloride incubated for 30 min. with autologous plasma. At the same time about 25 μCi ^{125}I human serum albumin was injected for determination of the plasma volume. Blood samples were taken from the opposite jugular vein at 5, 10, 15, 20, 25, 30 and 45 min. after injection and then at gradually lengthening intervals up to 3 hrs. (Fig. 3). Thereafter the blood samples were taken every second day to the 24th day to determine the erythrocyte ^{59}Fe uptake.

Radioactivity measurements were performed both on blood plasma and washed blood cells. The blood cells were washed 5 times with distilled water before measurement of radioactivity. Simultaneous measurements of ^{59}Fe and ^{125}I were performed in a 2 channel gamma counting system. The 1.098 and 1.289 MeV gamma emissions of ^{59}Fe could be measured without interference by ^{125}I . By subtracting the ^{59}Fe contribution to the ^{125}I peak at 0.035 MeV the amount of ^{125}I in the sample could be calculated.

The total plasma volume and the venous hematocrit were used to calculate the total blood volume at the beginning of the ferrokinetic study. Based on the relationship between plasma volume and body weight at that time, the plasma volume at the time of maximum erythrocyte ^{59}Fe uptake was calculated. The total blood volume was then estimated the actual hematocrit taken into consideration.

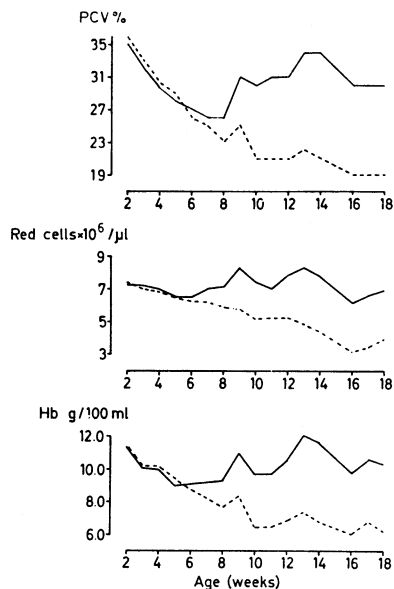


Figure 1. Hemoglobin (Hb), red cells and packed cell volume (PCV) in control (—) and experimental (---) calves from an age of 2 weeks to the end of the experiment. The ferrokinetic studies started at an age of about 15 weeks.

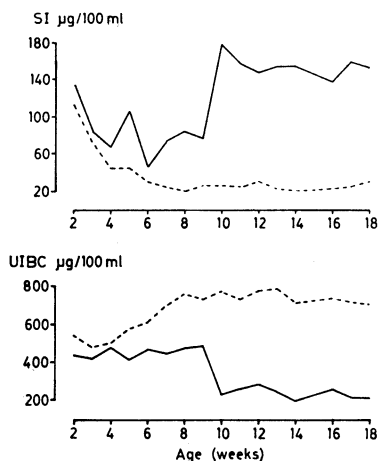


Figure 2. Unsaturated iron binding capacity (UIBC) and serum iron (SI) in control (—) and experimental (---) calves from an age of 2 weeks to the end of the experiment.

The plasma disappearance of ^{59}Fe during the first 45 min. after injection could be described satisfactorily by a single exponential function (Fig. 3). The time needed for half the injected dose to disappear from the plasma ($T_{1/2}$) was calculated according to the equation:

$$T_{1/2} = \frac{0.693}{k}$$

where 0.693 is the natural logarithm of 2.

The method of least squares was used to derive the line for each calf describing the plasma disappearance of ^{59}Fe from 5 up to 45 min. after injection. The following formulas were used (Marcilese *et al.* 1965):

a) $\text{TPI} = \text{SI} \times \text{TPV}$

TPI = total plasma iron (mg)

SI = serum iron ($\mu\text{g}/100 \text{ ml}$)

TPV = total plasma volume

b) $\text{TRCI} = \frac{\text{Hb} \times \text{TBV} \times 3.35}{100}$

TRCI = total red cell iron (g)

Hb = hemoglobin (g per 100 ml blood)

3.35 = mg of iron per g of Hb

TBV = total blood volume

c) $\text{PITR} = \frac{0.693 \times \text{TPI} \times 1440}{T_{1/2} \text{ } ^{59}\text{Fe}}$

PITR = plasma iron turnover rate (mg per day)

0.693 = ln of 2

1440 = minutes in 24 hrs.

$T_{1/2} \text{ } ^{59}\text{Fe}$ = time in minutes at which one half of the injected activity disappeared from the plasma

d) $\text{PIDRR} = \frac{\text{PITR}}{\text{TPI}}$

PIDRR = plasma iron daily renewal rate (times per day)

e) $^{59}\text{Fe Ut} = \frac{\text{RA in TRC} \times 100}{\text{TAI}}$

$^{59}\text{Fe Ut}$ = percentage of ^{59}Fe injected utilized by bone marrow to produce new red cells, calculated on day in which the curve of utilization reached its plateau
 RA in TRC = recovered activity in total red cells on day when cell activity reached its plateau
 TAI = total activity injected

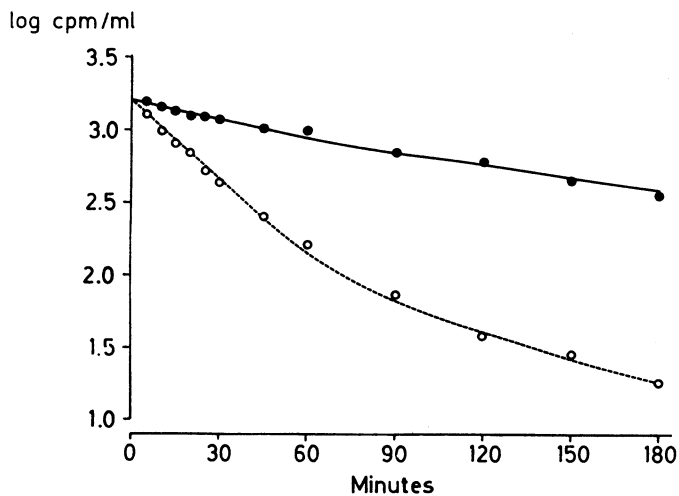


Figure 3. Disappearance of ^{59}Fe from blood plasma of 1 control (● --- ●) calf and 1 experimental (o --- o) calf of an age of 15 weeks. Half clearance times were determined from the first straight portion of each curve (the values from 5 to 45 min.).

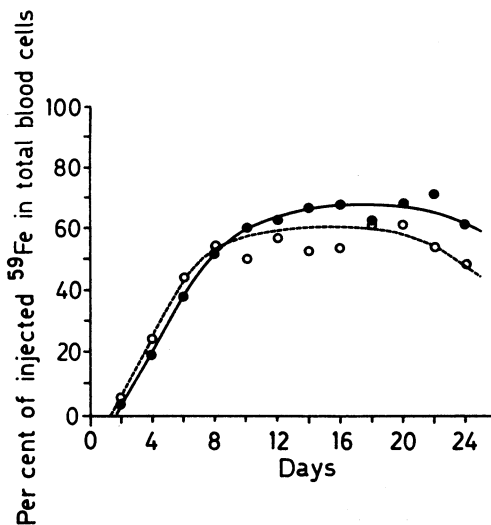


Figure 4. Uptake of ^{59}Fe into red cells of control (● --- ●) and experimental (o --- o) calves.

Provided that the amount of iron being liberated each day from destroyed red cells is the same as is incorporated into new red cells the amount of iron incorporated into new red cells to keep pace with growth can be determined (*Hansard 1965*):

$(\text{TBV day X} - \text{TBV day 0}) \times \text{Hb} \times 3.35 \times {}^{59}\text{Fe}$, where

TBV day X = the blood volume (ml) on the day when the maximum erythrocyte ${}^{59}\text{Fe}$ uptake is reached.

TBV day 0 = the blood volume when ${}^{59}\text{Fe}$ was injected.

Hb = the hemoglobin concentration (g/ml) on day X.

3.35 = the amount of iron in mg per gram of hemoglobin.

X = the number of days between injection and maximum concentration of ${}^{59}\text{Fe}$ in erythrocytes.

${}^{59}\text{Fe}$ max = the fraction of ${}^{59}\text{Fe}$ incorporated into red cell hemoglobin.

RESULTS

The development of the anemia in the experimental group and the hematological values of the control group can be studied in Figs. 1—2. There are pronounced differences between the mean values of the 2 groups at the time of the ferrokinetic study. The mean body weight values of the 2 groups were about the same (Table 2). One calf (no. 15) in the experimental group,

Table 2. Data used to calculate erythrokinetic values.

Calf no.	Body weight (kg)	Blood volume (ml)	Plasma volume (ml)	Plasma volume, % of b.w.	PCV (%)	Hb (g/100 ml)	SI* ($\mu\text{g}/100\text{ ml}$)	TPI** (mg)	TRCI*** (g)
12-C	88	5208	3437	3.9	44	14.3	149	5.12	2.49
13-C	98	5803	3946	4.0	32	10.6	83	3.28	2.06
52-C	107	7641	5578	5.2	27	9.3	140	7.81	2.34
54-C	110	6900	4692	4.3	32	10.3	165	7.74	2.37
Mean	101	6388	4413	4.4	34	11.1	134	5.99	2.32
11-E	104	6297	4849	4.7	23	6.9	21	1.02	1.45
14-E	102	7280	5533	5.4	24	7.7	19	1.05	1.87
15-E	83	5706	4508	5.4	21	6.8	19	0.86	1.30
16-E	106	6438	4958	4.7	23	7.4	13	0.65	1.59
Mean	99	6430	4962	5.1	23	7.2	18	0.90	1.55

SI* = Serum iron.

TPI** = Total plasma iron.

TRCI*** = Total red cell iron.

however, had a significantly lower body weight than the other calves in this group. The calves in the experimental group had somewhat higher plasma and blood volumes than the calves in the control group.

The plasma clearance of radio iron was more rapid in the experimental calves than in the controls (Fig. 3). The disappearance curves obtained both from control and experimental calves can be divided at least into 2 exponential parts, 1 corresponding to a short half clearance time and the other to a longer one (Fig. 3). The mean $T_{1/2}$ value calculated on the data obtained up to 45 min. after injection was about 4 times shorter in the experimental than in the control group (Table 3). The control calves showed a somewhat higher plasma iron turnover rate than the experimental ones. The mean values of the 2 groups were 0.85 and 0.53 Fe/kg/day, respectively.

Table 3. Erythrokinetic values in control and experimental calves.

Calf no.	$T_{1/2}^{59\text{Fe}}$ (min.)	PITR** (mg/kg/day)	PIDRR*** (times/day)
12-C	84	0.691	11.88
13-C	31	1.077	32.19
52-C	72	1.012	13.86
54-C	116	0.605	8.60
Mean	76	0.846	16.63
11-E	17	0.576	58.73
14-E	15	0.685	66.51
15-E	23	0.450	43.37
16-E	15	0.408	66.48
Mean	18	0.530	58.77

* $T_{1/2}^{59\text{Fe}}$ = Half clearance time of ^{59}Fe in plasma.

** PITR = Plasma iron turnover rate.

*** PIDRR = Plasma iron daily renewal rate.

The mean value of plasma iron daily renewal rate was about 3 times higher in the experimental than in the control group (Table 3).

The erythrocyte ^{59}Fe uptake for the 2 groups is shown in Fig. 4. The maximum value was reached on the 14th to 16th day after the administration of ^{59}Fe . The uptake of ^{59}Fe was about 10 %

higher in the control group than in the experimental. Calf no. 12 in the control group was not included in these comparisons, as it had a remarkably low uptake (about 28 %).

DISCUSSION

In the present study a pronounced anemia in the experimental group developed after 14 weeks on a commercial milk substitute diet. Lower hemoglobin concentration and erythrocyte values in the experimental group than in the control group were obvious after about 6 weeks on the milk substitute diet (Fig. 1). Low serum iron values as well as high unsaturated iron binding capacity (Fig. 2) indicate that the anemia was caused by iron deficiency.

At the time for the erythrokinetic study the calves in both groups had an average body weight of about 100 kg (Table 2). One would have expected a higher mean body weight in the experimental than in the control group because of the more intensive feeding of the experimental group. The very low body weight of calf no. 15 lowered the average weight of the experimental group. This calf had frequent attacks of enteritis and therefore was fed less than the others.

The plasma volume in relation to body weight was somewhat higher in the experimental group than in the control group. This difference can possibly be explained by the daily intake of large fluid volumes with the milk substitute. It should be pointed out, however, that the plasma volumes of the control calves were a little lower than has been previously reported (*Haxton et al.* 1974, *Möllerberg et al.* 1975 a).

In the ferrokinetic study, the mean control plasma ^{59}Fe clearance half-time is in accordance with values reported in calves 3 months old by *Baker & Douglas* (1957), but shorter than those reported by *Hansard et al.* (1959) and *Kaneko & Mattheeuws* (1966). However, these calves were older than in the present investigation. Because they based their calculations of plasma clearance half-time on ^{59}Fe values at up to 3 and 2 hrs., respectively, after injection, their $T_{1/2}$ values were higher. The $T_{1/2}$ values in the anemic group were about 25 % of those in the normal calves. That is in good agreement with the results obtained in anemic and normal humans (*Bothwell & Finch* 1962) as well as in anemic and normal piglets (*Ekman & Iwanska* 1966). It is interesting to note that the very low plasma iron concentration

in the anemic calves is partially compensated by a more rapid plasma iron disappearance rate so that the difference in the plasma iron turnover rate between the calves is reduced. The plasma iron turnover rate is considered to be an indirect measurement of the erythroblastic activity of the bone marrow (*Bothwell et al.* 1957).

The plasma iron daily renewal rate was about 3 times higher in the anemic group than in the normal, and is a compensatory process to reduce the effect of lower total plasma iron.

The maximum erythrocyte ^{59}Fe uptake was reached about 14 to 16 days after injection. This is in accordance with results reported by *Hansard et al.* and *Kaneko & Mattheeuws*, but a somewhat longer time than *Baker & Douglas* found in 3 months old calves. The uptake of about 70 % in the normal calves is identical with results in studies by *Baker & Douglas* and *Kaneko & Mattheeuws*.

The appearance of injected radio iron in circulating red cells in connection with other measurements such as serum iron, hemoglobin, number of red cells and packed cell volume is considered to be a measure of bone marrow function (*Bothwell & Finch*).

One would have expected a higher erythrocyte ^{59}Fe uptake in the anemic calves than in the controls. That was not the case, because the iron storage was greatly reduced as indicated by the low serum iron values. Consequently the formation and transfer of new red cells into circulation was retarded. It should be noted that during the ferrokinetic study the total red cell mass in the anemic calves decreased despite an average increase in body weight of 5.5 kg.

A low erythrocyte ^{59}Fe uptake such as occurred in calf no. 12 has been seen in conditions such as marrow hypoplasia, ineffective erythropoietic activity and iron overload (*Bothwell & Finch*). As this calf had higher Hb and PCV values than the other control calves, it probably did not have decreased marrow function. The reason for the low ^{59}Fe uptake in this calf is unclear.

The greater part of the calf's iron requirement is used for growth (*Matrone et al.* 1957). To calculate the iron requirement of calves for growth we can use as an example calf no. 13 with normal blood values and normal growth rate. This calf had unchanged Hb and PCV values from the day when ^{59}Fe was injected to the 14th day and had gained 5 kg in weight during that

period. The blood volume had increased during this time by 256 ml. To maintain a constant Hb level of 10.6 g/100 ml and to increase the blood volume by 256 ml, the iron requirement can be calculated using the previously mentioned formula:

$$256 \times 0.106 \times 3.35 \times 0.70 = 63.6 \text{ mg Fe}$$

To increase the blood volume by 1 ml the requirement is:

$$\frac{63.6}{256} = 0.25 \text{ mg Fe}$$

According to Möllerberg *et al.* (1975 a) the blood volume of calves weighing about 100 kg is about 70 ml per kg body weight. Accordingly the iron requirement for 1 kg growth and maintaining a constant Hb of 10.6 can be calculated: $70 \times 0.25 = 17.5$ mg Fe. These calculations are made on the assumption that the rate of production of red cells is equal to the rate of their destruction. The iron requirement for hemoglobin synthesis estimated in this study is in accordance with what Blaxter *et al.* (1957) reported, 19 mg Fe per kg gain. Blaxter *et al.* have estimated the Fe contained in enzymes and myoglobin to be approx. 20 mg/kg body weight. The total requirement of a calf gaining 1 kg daily excluding any need to store iron in the liver or extra hepatic tissue will thus be about 35–40 mg/day.

The liver and the spleen are regarded as the most important storage organs for iron. Liver non-hem. iron levels in calves are reported to be about 5 mg/100 g fresh weight (Niedermeier *et al.* 1959, Hibbs *et al.* 1963, Kirchgessner *et al.* 1971). Kolb (1963) reports the spleen non-hem. iron to 69.3 mg/100 g dry substance, corresponding to about 20 mg Fe/100 g fresh weight. The average weights of the liver and spleen of calves between 92 and 130 kg are 1.93 and 0.34 % of body weight, respectively (Nilsson 1974).

It appears from the above calculations that the total requirements of a 100 kg calf gaining 1 kg daily will be between 40–45 mg/day, assuming a complete absorption and retention of dietary iron. The absorption, however, is normally far from complete, but will vary with the iron nutrition state, the chemical form of iron in feed, and the composition of the feed. Assuming a maximal retention of dietary iron to be 25 %, a calf weighing 100 kg and gaining 1 kg daily will need 160 to 180 mg.

REFERENCES

- Baker, N. F. & J. R. Douglas*: Pathogenesis of trichostrongyloid parasites. II. Ferrokinetic studies in ruminants. *Amer. J. vet. Res.* 1957, **18**, 295—302.
- Blaxter, K. L., G. A. M. Sharman & A. M. Mc Donald*: Iron deficiency anemia in calves. *Brit. J. Nutr.* 1957, **11**, 234—246.
- Bothwell, T. H. & C. A. Finch*: Iron Metabolism. Churchill, London, Little Brown & Company, Boston 1962.
- Bothwell, T. H., A. V. Hustado, D. M. Donohue & C. A. Finch*: Erythrokinetics. IV. The plasma iron turnover as a measure of erythropoiesis. *Blood* 1957, **12**, 409—427.
- Ekman, L. & S. Iwńska*: Studies in iron metabolism in normal and anemic nursing pigs. *Zbl. Vet.-Med. A.* 1966, **13**, 585—595.
- Hansard, S. L.*: Radioisotopes in animal nutrition and physiology. IAEA Symp., Vienna 1965, 319—337.
- Hansard, S. L., L. E. Foote & G. Dimopoulos*: The physiological behavior of iron in the calf. *J. Dairy Sci.* 1959, **42**, 1970—1976.
- Haxton, J. A., M. D. Schneider & M. P. Kaye*: Blood volume of the male Holstein-Friesian calf. *Amer. J. vet. Res.* 1974, **35**, 835—837.
- Hibbs, J. W., H. R. Conrad, J. H. Vandersall & C. Gale*: Occurrence of iron deficiency anemia in dairy calves at birth and its alleviation by iron dextran injection. *J. Dairy Sci.* 1963, **46**, 1118—1124.
- Kaneko, J. J. & D. R. G. Mattheeuws*: Iron metabolism in normal and porphyric calves. *Amer. J. vet. Res.* 1966, **27**, 923—929.
- Kirchgessner, M., E. Grassman, J. Krippel & H. L. Müller*: Zum Einfluss einmaliger und kontinuierlicher Fe-Zulagen in der Kälbermast auf ernährungsphysiologische Wirksamkeit und Fleischfarbe. (On the influence of unique and continuous Fe supplementation in calf fattening on nutritional efficaciousness and meat colour). *Züchtungskunde* 1971, **43**, 336—345.
- Kolb, E.*: Iron metabolism in farm animals. *Adv. vet. Sci.* 1963, **8**, 49.
- Marcilese, N. A., H. D. Figueiras, R. M. Valsecchi, A. H. Fraga, H. R. Camberos & J. E. Varela*: Erythrokinetics in the horse. *Amer. J. Physiol.* 1965, **209**, 727—730.
- Matrone, G., C. Conley, G. H. Wise & R. K. Waugh*: A study of iron and copper requirements of dairy calves. *J. Dairy Sci.* 1957, **40**, 1437—1447.
- Matzen, K.*: Blutuntersuchungen bei Mastkälbern. (The hematological picture of veal calves). Thesis, München 1968.
- Möllerberg, L.*: A hematologic and blood chemical study of Swedish purchased calves. *Acta vet. scand.* 1975, **16**, 170—177.
- Möllerberg, L., L. Ekman & S.-O. Jacobsson*: Plasma and blood volume in the calf from birth till 90 days of age. *Acta vet. scand.* 1975 a, **16**, 178—185.
- Möllerberg, L., T. Ehlers, S.-O. Jacobsson, S. Johnsson & I. Olsson*: The effect of parenteral iron supply on hematology, health, growth

- and meat classification in veal calves. *Acta vet. scand.* 1975 b, 16, 197—204.
- Niedermeier, R. P., N. N. Allén, R. D. Lance, E. H. Rupnow & R. W. Bray: Effect of feeding methods on veal production and carcass quality. I. Rate of gain, stomach capacity, vitamin A, iron and hemoglobin values. *J. Animal Sci.* 1959, 18, 726—731.
- Nilsson, K.: Personal communication 1974.
- Scheidegger, H. P.: Veränderungen des roten Blutbildes und der Serum-eisen Konzentration bei Simmentaler Kälbern. (Red cells and serum iron values in Simmertaler calves). *Schweiz. Arch. Tierheilk.* 1973, 115, 483—497.
- Tollerz, G.: Studies on the tolerance to iron in piglets and mice with special reference to vitamin E, synthetic antioxidants and sodium-selenite. Thesis, Stockholm 1965.
- Weinberg, E. D.: Iron and susceptibility to infectious disease. *Science* 1974, 184, 952—956.

SAMMANFATTNING

Ferrokinetiska studier hos normala kalvar och kalvar med järnbristanemi.

En studie genomfördes över järnmetabolismen hos normala kalvar och kalvar med experimentellt framkallad järnbristanemi.

Eliminationshastigheten av ^{59}Fe från blodplasma var omkring fyra gånger kortare hos anemiska kalvar än hos kontrollkalvar. En låg koncentration av plasmajärn hos anemiska kalvar kompenserades delvis genom en accelererad järnelimination varför skillnaden i „plasma iron turnover rate“ reducerades.

Maximala upptaget av ^{59}Fe i de röda blodkropparna erhöles 14 till 16 dagar efter injektion. Upptaget av ^{59}Fe var ca 10 % högre hos kontrollkalvarna än hos de anemiska kalvarna.

Med ledning av de ferrokinetiska studierna beräknades järnbehovet för växande kalvar. En kalv som växer ett kg och väger omkring 100 kg kräver 17,5 mg Fe för att uppehålla ett konstant och normalt Hb-värde. Med ledning av litteraturuppgifter beräknades det totala dagliga behovet för en sådan kalv vid en tillväxt av 1 kg/dag till 160—180 mg. Därvid har antagits att 25 % av järnet i fodret resorberas och retineras.

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