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Measurement of iron absorption in milk-fed calves using a dual stable isotope technique*

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ABSTRACT

Fractional absorption of an oral dose of iron was determined in calves receiving an iron-deficient diet with and without supplementation of iron. Iron supplements were Fe-equivalent doses of oral ferrous sulphate or parenteral gleptoferron. Animals receiving no supplementation showed haematological results suggestive of mild anaemia. Forms of iron supplementation did not differ significantly in their ability to prevent the onset of anaemia. Iron fractional absorption determined using a dual stable isotope labelling method was 60, 39 and 18% in the iron deficient calves, and the calves supplemented with parenteral iron and oral iron, respectively.

KEY WORDS: iron, absorption, calves, stable isotopes

INTRODUCTION

Dual stable isotopic labelling has been used to measure the fractional absorption of minerals in humans (e.g., Kastenmayer et al., 1994; Walczyk et al., 1997). We have adapted this technique in the development of a pre-ruminant model to study the effects of oral vs parenteral supplementation on the regulation of iron metabolism.

MATERIAL AND METHODS

Eighteen pre-ruminant calves from 2 sires were used to develop an animal model of contrasting states of iron absorption over an experimental period of 68 d. The diet consisted of 100% reconstituted milk powder (increasing concentration: 14 to 20% milk solids; 1.7 mg Fe/kg DM). The calves were 8.7 (SD 2.6) days

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old and 43.7 (SD 5.7) kg liveweight (LW) when treatments started. Treatments consisted of no Fe supplementation (Fe-deficient), Parenteral Fe (600 mg of iron as gleptoferron: Gleptosyl™ intramuscular every 21 d) and Oral Fe (100 mg of Fe as iron sulphate per kg milk solids). The two Fe treatments were planned to provide the equivalent of 34 mg of physiologically available Fe per kg of LW gain. Blood samples were collected weekly and LW of the calves recorded twice weekly. Blood samples were analysed for haematological parameters using a Coulter Counter. Iron status of the calves was assessed using haemoglobin (Hb) concentrations in blood. On d 47, blood volumes were estimated using the Evan's Blue dilution method and haematocrit to estimate the mass of the blood Fe pool and calculate optimal loads of iron isotopes. On d 48, four animals in each treatment group were dosed with ^{57}Fe (oral) and ^{58}Fe (intravenous). A week later (d 55), erythrocytes were collected and analysed for iron isotope concentrations by dynamic reaction cell ICP-MS. Ratios of $^{57}Fe/^{58}Fe$ in blood cells were used to calculate the fractional absorption of orally-dosed iron. Animals were slaughtered on d 62 and 63 of the experiment (n=9 each d), liver and *longissimus dorsi* muscle collected and analysed for total iron concentration.

Statistical analysis was conducted using the GLM and MIXED procedures of SAS v 8.0 (Hb concentration data were analysed using the "repeated" option with auto-regressive variance-covariance structure and 'time' effect). Treatment, sire and their interaction were included as main effects. All animal manipulations were approved by the Crown Research Institutes Palmerston North Campus Animal Ethics Committee.

RESULTS

Overall daily LW gain was not affected by treatments (0.7 kg/d). Plasma volumes (% of LW) measured on day 47 were 8.2 (range 6.6 to 10.3), 9.9 (range 6.0 to 16.9) and 8.8 (range 6.4 to 15.4) for the Control, Parenteral Fe and Oral Fe, respectively. Actual iron intake was 40 and 28 mg Fe per kg LW gain for the parenteral and oral iron groups, respectively.

There were no significant differences in the blood Hb concentration of calves receiving parenteral or oral iron supplementation. Clinical data from weekly blood samples showed that the Fe-deficient group developed signs compatible with low iron status (mild hypochromic anaemia: Figure 1). Significant differences between the Fe-deficient and Fe supplemented groups were detected as early as 28 d of the experimental period.

Fe deficiency resulted in greater dietary Fe absorption compared to Fe-supplemented animals (P=0.02). Iron deficient calves had a lower hepatic Fe concentration (P=0.01) that was 64 and 17% of those measured in orally- and parenterally-supplemented calves, respectively). Fe-deficient calves had lower Fe

Fe-deficient vs Parenteral Fe	NS	NS	NS	NS	NS	NS	NS	*	*
Fe-deficient vs Oral Fe	NS	NS	NS	*	**	**	**	**	**

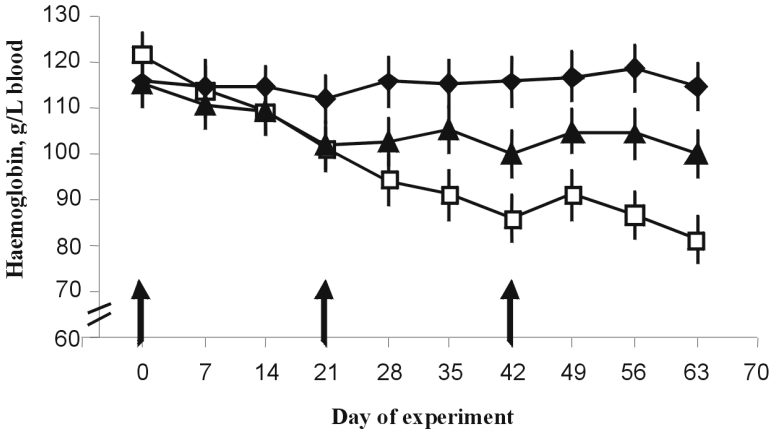

Figure 1. Haemoglobin concentration in blood of calves receiving three regimes of iron supplementation. Symbols indicate means ± SEM (n=6). NS: Not significant, *P<0.05, **P<0.01. Arrows indicate the times of parenteral iron dosage. □: Fe-deficient, ▲: Parenteral Fe, ◆: Oral Fe

muscular concentration (P=0.01) that was 71 and 75% of the concentrations in Oral Fe and Parenteral Fe groups, respectively (Table 1).

Table 1. Fractional absorption of iron and hepatic iron concentration in calves receiving three iron regimes. Means with different letter within column are different (LSD α = 0.01)

Treatment	Fe absorption, %[1]		Fe in tissues, µg Fe/g tissue[2]			
			liver		muscle	
	mean	range	mean	range	mean	range
Fe-deficient	60[a]	28-87	52[a]	23-111	5.5[a]	3.2-8.2
Parenteral Fe	39[ab]	30-52	296[b]	176-722	7.3[ab]	4.8-9.9
Oral Fe	18[b]	12-24	81[ab]	40-163	7.7[b]	6.6-8.4

[1] n=4 for Fe-deficient and oral Fe and n=3 for parenteral Fe; [2] wet basis n=6

DISCUSSION

To our knowledge, this is the first report of fractional absorption of Fe measured in calves using the dual stable isotope labelling method. Our results confirm previous indirect estimates of absorption in calves consuming Fe-deficient diets or receiving oral Fe-supplementation with contrasting Fe status (NRC, 2001 and references therein).

Although the parenteral and oral Fe dose rates were equivalent on a LW gain basis, it appears that the injected amount may have been just sufficient to

maintain adequate iron homeostasis. This is indicated by a significant drop in Hb concentration towards the end of each 21-d period between administration of parenteral Fe (d 42: $P=0.05$ and d 63: $P=0.06$). The hepatic Fe concentration measured at the end of the experiment (d 63) suggested that the body appeared to manage the higher Fe load of parenteral dose by storing it in the liver, presumably as inert haemosiderin. The difference in mean and range in fractional absorption in the Fe-supplemented animals suggested that the parenteral administration of Fe is not as effective as its oral equivalent in establishing a feedback signal to the gut to reduce the fractional absorption of Fe from the diet.

CONCLUSIONS

The dual stable isotope method for estimation of fractional absorption of iron provides sensible estimates of iron absorption in milk-fed calves. While parenteral iron seems adequate to prevent signs of sub-clinical anaemia, there is indication that the body stores a sizeable proportion of the parenteral dose in the liver, with incomplete feedback to the gastrointestinal tract. Given the clear functional difference in Fe absorption, the animal model described herein appears suitable to assess the effect of iron supplementation on the genome and proteome involved in Fe absorption in the pre-ruminant animal.

REFERENCES

Kastenmayer P., Davidsson L., Galan P., Cherouvrier F., Hercberg S., Hurrell R.F., 1994. A double isotope technique for measuring iron absorption in infants. Brit. J. Nutr. 71, 411-424

National Research Council, 2001. Nutrient Requirements of Dairy Cattle. 7th Edition. National Academy Press, Washington, DC

Walczyk T., Davidsson L., Zavaleta N., Hurrell R.F., 1997. Stable isotope labels as a tool to determine the iron absorption by Peruvian school children from a breakfast meal. Fresenius J. Anal. Chem. 359, 445-449