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Dietary goat milk improves iron bioavailability in rats with induced ferropenic anaemia in comparison with cow milk

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Abstract

Using rats with induced iron (Fe)-deficiency anaemia, this study investigated the effects of diets based on goat milk (GM) or cow milk (CM) lyophilates on the nutritive utilization of Fe, its deposit in target organs and haematic parameters involved in Fe metabolism. GM improved Fe metabolism, especially in Fe-deficient rats, leading to a higher Fe content in the spleen, liver, sternum and femur in comparison with CM. After feeding the rats for 2 weeks with the different diets, the anaemia had decreased, especially with GM, as assessed by higher haemoglobin regeneration efficiency (HRE), serum Fe, red blood cells (RBC) and packed cellular volume (PCV) levels and lower platelet count. We conclude that dietary GM improves Fe bioavailability in both control and anaemic rats, increasing Fe deposits in target organs and favouring the recovery of haematological parameters after ferropenic nutritional anaemia.

Keywords: Goat milk; Cow milk; Iron bioavailability; Anaemia; Rats

1. Introduction

Anaemia is defined as a pathological process in which the haemoglobin (Hb) concentration in red cells is abnormally low. There is no doubt that iron deficiency (ID) is the cause of most forms of anaemia. Iron deficiency anaemia (IDA) is characterized by the reduction or absence of iron (Fe) stores, low serum concentration of Fe, poor Hb concentration, haematocrit reduction and increased platelet count. This type of anaemia is particularly prevalent in developing countries (Viteri, 1993) and, due to its effects on development and growth, resistance to infections and association with the mortality of infants younger than 2 years, is considered the major public health problem and the most common nutritional deficiency in the world. Moreover, ID has negative effects on work capacity and on motor and mental development in infants, children, adolescents, fertile and pregnant women and the elderly (Grantham-McGregor & Ani, 2001; Haas & Brownlie, 2001). To prevent ID, vulnerable populations should be encouraged to consume Fe-rich foods and breast-feed or use Fe-fortified formula for infants. Milk and other dairy products obtained from cows, being rich in calcium, interfere with the absorption of Fe from the diet. However, recent studies have shown that when goat milk (GM) is incorporated into the diet of rats, it produces a greater nutritive use of Fe (Barrionuevo, Alférez, López-Aliaga, Sanz Sampelayo, & Campos, 2002) and minimizes the possible interactions of Fe with other minerals such as Ca, P and Mg, in comparison with animals fed with cow milk (CM) (López-Aliaga, Alférez, Barrionuevo, Lisbona, & Campos, 2000).

Taking these considerations into account, the aim of the present study was to determine the effects of the particular nutritional characteristics of GM on the digestive and metabolic use of Fe, on haematic parameters and on the distribution of Fe in various target organs[1] of rats with ferropenic anaemia (compared with a control group) to

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[1]For simplicity, use of the term "organ" includes femur and sternum.

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assess the bioavailability of this mineral in comparison with the corresponding effects produced by a diet including CM (the most commonly consumed milk).

2. Materials and methods

2.1. Animals

Male Wistar albino breed rats ($n = 59$) recently weaned, aged ~3 weeks, purchased from the University of Granada Laboratory Animal Service were used for this study. Animal care procedures and experimental protocols were approved by the Ethics Committee of the University of Granada in accordance with the European Community guidelines.

2.2. Experimental design and diets

Fig. 1 shows the experimental design of the study. After weaning, the rats were randomly divided into two groups. Dietary Fe deficiency was induced in one group as previously described (Pallarés, Lisbona, López-Aliaga, Barrionuevo, Alférez, & Campos, 1993). Briefly, the rats underwent a pre-experimental period (PEP) of 40 d, during the first 10 d the rats were fed with the AIN-93G diet for growth (low in Fe: 5 mg kg^{-1} diet) following of AIN-93M diet for adult maintenance (low in Fe: 5 mg kg^{-1} diet) for 30 d (Reeves, Nielsen, & Fahey, 1993). The low-Fe diets used to induce anaemia in the rats were obtained by omitting Fe from the mineral supplement of each of the American Institute of Nutrition (AIN)-93 diets. During the PEP, the control group received the same diets but with a Fe-normal content (45 mg kg^{-1} Fe diet). After the PEP (day 0 of the experiment period, EP) both the anaemic and the control rats were divided into three groups, which were fed for 14 d with three different diets with a normal Fe content: standard diet (S), CM-based diet (C) and GM-based diet (G).

From the start of the study, the animals were placed in individual metabolic cages in an environmentally controlled room with a constant temperature of 20–22 °C, a 12 h light–dark cycle and 55–60% humidity. Diet and mineral-free water was available ad libitum to all rats. During the EP, food intake was measured and urine and faeces were collected daily. The faeces were lyophilized (FTS System, Inc., TDS-3, New York, USA), weighed and homogenized (Moulinex grinder 980, Groupe Seb Ibérica, Barcelona, Spain). Urine was collected in a 0.5% v/v HCl solution and later filtered (Whatman No. 41 filter papers, ashless; Whatman, Maidstone, UK). Body weight was

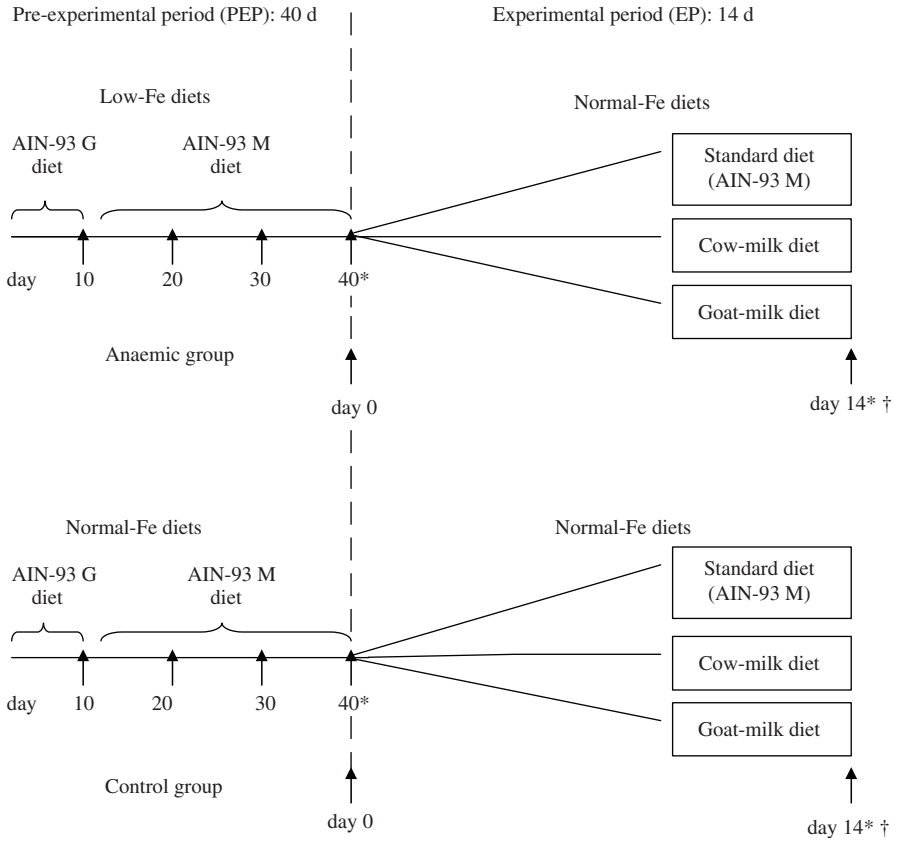

Fig. 1. Experimental design of the study. After a pre-experimental period of 40 d to obtain an anaemic rats group, the animals were allocated to one of the three normal-Fe diets (standard, cow-milk and goat-milk). *Blood from caudal vein was withdrawn on day 40 of the PEP (day 0 of experimental period) to ensure that the anaemia had been reached and on day 14 of the EP to determine haematological parameters. †Blood from abdominal aorta was withdrawn on day 14 of the EP to obtain serum, and the rats were euthanazed to remove the liver, femur, spleen and sternum. For details of diets, see Table 1.

recorded at the beginning and end of the PEP and EP. On day 40 of the PEP (day 0 EP) and day 14 of the EP, blood was collected from the caudal vein after an overnight period of food deprivation (12 h). On day 14 of the EP, the rats, after fasting overnight, were weighed and anesthetized by intraperitoneal injection of 5 mg 100 g^{-1} body weight of sodium pentobarbital (Sigma, St Louis, MO, USA). After median laparotomy, the rats were totally bled by cannulation of the abdominal aorta; the liver, both femurs, the spleen and the sternum were removed, washed with ice-cold saline solution (0.9%, w/v, NaCl) and stored immediately at −40 °C until Fe analysis. Serum was separated by centrifugation at 1500 × *g* for 15 min at 4 °C and stored at −40 °C.

Table 1 shows the chemical composition of the goat and cow milk lyophilates, used in producing the diets. Table 2 summarizes the different diets assayed during the PEP and EP. The diets and mineral and vitamin supplements provided during the EP were prepared according to the recommendations of the AIN (Reeves et al., 1993), except for the level of protein (20% instead of 14%) and the source and level of fat (10% rather than 4%). The standard diet (diet S) was prepared using virgin olive oil as the source of fat and casein as the protein source. The

Table 1
Average composition of lyophilates (100 g) from goats' and cows' milk[a]

	Goat milk	Cow milk
Protein (g)	23.36	24.84
Lipids (g)	30.69	28.76
Lactose (g)	39.20	40.75
Ca (mg)	1319	1030
P (mg)	813	782
Mg (mg)	89.5	85.2
Fe (mg)	1.23	0.87
Cu (mg)	0.25	0.14
Zn (mg)	4.1	3.5
Vitamin C (mg)	14.1	10.3
Vitamin A (mg)	0.43	0.24
Vitamin D (μg)	0.74	0.50
Saturated fatty acids (g)		
C4	0.98	0.83
C6	0.36	0.25
C8	0.65	0.26
C10	2.08	0.64
C12	0.78	0.59
C14	2.70	2.73
C16	7.57	7.35
C18	3.18	2.89
Monounsaturated fatty acids (g)		
C16:1	1.30	1.27
C18:1	8.46	7.23
Polyunsaturated fatty acids (g)		
C18:2	0.71	0.52
C18:3	0.18	0.19
Amino acids (g)		
Alanine	0.90	0.87
Arginine	0.91	0.90
Aspartic acid	1.75	1.87
Cystine	0.32	0.21
Glutamic acid	5.23	5.47
Glycine	0.48	0.53
Histidine	0.61	0.62
Isoleucine	1.64	1.58
Leucine	2.28	2.39
Lysine	2.14	1.93
Methionine	0.60	0.61
Phenylalanine	1.18	1.20
Proline	2.75	2.39
Serine	1.42	1.41
Threonine	1.24	1.14
Tyrosine	1.38	1.23
Valine	0.10	0.94

[a]The data presented are means ± standard deviation (SD) for three replicate trials.

Table 2
Composition of the experimental diets (g kg^{-1} diet)

Component	Amount
Pre-experimental period	
AIN-93G diet[a]	
Casein	200
Olive oil	70
Wheat starch	530
Constant ingredients[b]	200
AIN-93M diet[a]	
Casein	140
Olive oil	40
Wheat starch	621
Constant ingredients[b]	199
Experimental period	
Diet S (non-milk standard)[c]	
Casein	200
Olive oil	100
Wheat starch	501
Constant ingredients[d]	199
Diet C (cow milk)[c]	
Casein + protein cow milk	200
Cow milk fat	100
Wheat starch	307
Lactose	194
Constant ingredients[d]	199
Diet G (goat milk)[c]	
Casein + protein goat milk	200
Goat milk fat	100
Wheat starch	311
Lactose	199
Constant ingredients[d]	199

[a]Normal-Fe (45 mg kg^{-1} diet) and low-Fe (5 mg kg^{-1} diet).

[b]The constant ingredients consisted of (g kg^{-1} diet): fibre (micronized cellulose), 50; sucrose, 100; choline chloride, 2.5; L-cystine, 3 for growing and 1.8 for adult rats; mineral premix, 35; vitamin premix, 10. The mineral and vitamin premixes were prepared according to the recommendations of the American Institute of Nutrition (1993) for growing and adults rats.

[c]Normal-Fe (45 mg kg^{-1} diet).

[d]The constant ingredients consisted of (g kg^{-1} diet): fibre (micronized cellulose), 50; sucrose, 100; choline chloride, 2.5; L-cystine, 1.8; mineral premix, 35; vitamin premix, 10. The mineral and vitamin premixes were prepared according to the recommendations of the American Institute of Nutrition (1993) for standard diet and mineral and vitamin specific supplements for diets C and G were formulated taking into account the mineral content of the lyophilized milks supplied in order to meet these recommendations.

milk-based diets were made with lyophilized cow or goat milk (diets C and G, respectively). The necessary quantities of lyophilized cow or goat milk were taken into consideration to obtain a diet with a 10% fat content. To obtain the 20% protein content, the diet was supplemented with casein (Musal & Chemical, Granada, Spain), with 124 g of casein kg^{-1} for diet C and 145 g of casein kg^{-1} for diet G, as the protein provided by the lyophilate used for the milk-based diets was insufficient. The Fe content in the diets used during the EP after analysis was as follows: diet S, 46.09; diet C, 44.71; and diet G, 44.14 (mg kg^{-1} diet).

2.3. Biological indices

The following indices were calculated from the data on Fe intake and faecal and urinary Fe excretion, according to the formulas given below: intake (expressed as dry weight), body weight, apparent digestibility coefficient (ADC, Eq. (1)) and retention for Fe (balance, Eq. (2)) and per cent Fe retention/Fe intake (% R/I, Eq. (3))

$$\mathrm{ADC} = (I - F) \times 100/I, \tag{1}$$

$$\text{Balance (retention)} = I - (F + U), \tag{2}$$

$$\%R/I = I - (F + U) \times 100/I, \tag{3}$$

where I = intake, F = faecal excretion and U = urinary excretion.

Haemoglobin regeneration efficiency (HRE) was calculated as previously described (Zhang, Hendricks, & Mahoney, 1989):

Hb-Fe (mg) = body weight (g) × mL blood g^{-1} body weight (assumed to be 0.067 mL) × g Hb mL^{-1} blood × mg Fe g^{-1} Hb (assumed to be 3.35 mg).

$$\text{Percentage HRE} = \frac{\text{mg Hb-Fe (final)} - \text{mg Hb-Fe (initial)}}{\text{mg Fe consumed}} \times 100.$$

2.4. Analytical methods

All reagents were of analytical grade, and ultrapure water of 18 MΩ cm specific resistivity was obtained from a Milli-Q purification system (Millipore Corp., MA, USA).

2.4.1. Dry matter

The water contents of the diets, faeces, liver, sternum, femur and spleen were determined by drying the materials at 105 ± 2 °C to constant weight.

2.4.2. Iron determination

The concentrations of Fe in the diet, faeces, urine and the different organs were determined by atomic absorption spectrophotometry (Perkin-Elmer 1100B, Shelton, CT, USA). The samples had been previously mineralized by a wet method in a sand bath (J.R. SELECTA, Barcelona, Spain), placing the samples in a resistant flask and dissolving using nitric acid, followed by a mixing with HNO_3:$HClO_4$ (1:4, v/v) until the total elimination of organic matter. Finally, the samples were diluted with Milli-Q water and filtered through a Whatman No. 41 filter. Samples of lyophilized bovine liver (certified reference material CBR 185; Community Bureau of References, Brussels, Belgium) were simultaneously used to check the Fe recovery (Fe value = 210 ± 6 mg kg^{-1}, mean ± SD of five determinations, certified value 214 ± 5 mg kg^{-1}). All glassware and the polyethylene samples bottles used were washed using 10 N nitric acid, and demineralized water (Milli-Q).

2.4.3. Compositional analyses

Nitrogen content in the lyophilates and diets was determined by the Kjeldahl method, using a protein conversion factor of 6.38. The amino acid composition of the proteins was determined by high-performance liquid chromatography using the Waters Pico-Tag method with precolumn derivatization and phenylisothiocyanate as previously described (Nestares, López-Frías, Barrionuevo, & Urbano, 1996).

Fat content in the lyophilates and diets was analysed by the gravimetric method of Weibull and Berntrop (1988). The fatty-acid patterns of the lyophilates were determined by gas-liquid chromatography (GLC) using the method of Lepage and Roy (1986).

The vitamin A and D_3 (cholecalciferol) content of the lyophilates was measured by HPLC according to the method of Heudi, Trisconi, and Blake (2004). The vitamin C content of the lyophilates was measured by a colorimetric method using a commercial kit (Boehringer Mannheim, Germany).

Hb concentration, red blood cells (RBC), packed cellular volume (PCV) and platelets of fresh, heparinized blood samples were measured using Sysmex K-1000D (Automated Haematology Analyzer, Holliston, Massachusetts, USA). Serum levels of Fe were determined by colorimetry (Trinder, 1956).

2.5. Statistical analysis

Data are reported as means with standard errors. The data were analysed by two-way ANOVA using a model with two main effects (animal group and type of diet). Following a significant F test ($P<0.05$), post hoc Tukey's test was used to determine significant differences between means. Pearson correlation coefficients between selected individual measurements were calculated. All statistical analyses were carried out using a SPSS software package (SPSS, 2004, version 12.0).

3. Results

3.1. Digestive and metabolic utilization of iron

Two-way ANOVA revealed that Fe intake was affected by the anaemia ($P<0.05$) and the diet type ($P<0.001$),

therefore Fe intake was significantly higher in the Fe-deficient rats that consumed diet S than in the control group ($P<0.01$). However, Fe intake was similar among the control and anaemic rats given the two milk-based diets (C and G). Fe intake was affected by the diet ($P<0.001$), being higher in both groups of rats that consumed the standard diet with respect to the milk-based diets ($P<0.001$). Among the control rats Fe intake was similar for the two milk-based diets. However, in anaemic rats Fe intake was higher among the rats fed with the CM diet than among those fed with diet G ($P<0.01$) (Fe intake: Controls S > C = G; Anaemics S > C > G) (Table 3).

The digestive use (ADC) of Fe was affected by the diet and by anaemia (two-way ANOVA, $P<0.001$). The ADC of Fe was greater for the Fe-deficient rats than for their controls ($P<0.001$), regardless of the type of diet consumed. Comparison of the effects of the three diets revealed that among the controls and the Fe-deficient rats, the ADC of Fe was greater when the animals were given diet G than when given diets S or C, which were similar (G:S, $P<0.01$; G:C, $P<0.05$ for the control rats and G:S and G:C, $P<0.001$ for the anaemic rats) (ADC of Fe: G > S = C) (Table 3).

The Fe balance was increased by anaemia (two-way ANOVA, $P<0.001$) and also, though only slightly, by the diet (two-way ANOVA, $P<0.05$). Higher levels of Fe retention were obtained for anaemic rats in comparison with the controls, for all the diets studied ($P<0.001$). Moreover, the Fe retention/intake was affected by the diet and by anaemia (two way ANOVA, $P<0.001$), with higher values being obtained for diet G than among the rats given the other two diets, in both the control animals ($P<0.01$ for G:S and $P<0.05$ for G:C) and the anaemic rats ($P<0.001$ for G:S and G:C) (R/I of Fe: G > S = C) (Table 3).

Two-way ANOVA indicated that there was not significant interaction between diets × anaemia for the different variables related to the digestive and metabolic utilization of Fe.

3.2. Iron content of rat organs

In general, the Fe content was lower in all the organs studied (spleen, liver, sternum and femur) in the anaemic rats in comparison with their controls, for the three diets assayed ($P<0.001$), except in the sternum (controls = anaemics) for diet S. Two-way ANOVA showed that both the diet and the anaemia had a significant effect on Fe deposits in organs, except for that of diet on deposits in the spleen. In addition, an interaction effect between diet and anaemia was observed ($P<0.001$) in the Fe concentration in the femur and the sternum. Comparison of the milk-based diets revealed that the Fe content in the organs was higher for both groups of animals fed with diet G, particularly in the sternum and femur ($P<0.001$) for the controls rats, and in the spleen ($P<0.025$), sternum ($P<0.01$) and femur ($P<0.001$) for the anaemic rats.

Table 3
Digestive and metabolic utilization of iron in control and iron-deficient rats fed different diets[a]

	Standard diet		Cow milk diet		Goat milk diet		Two-way ANOVA[b] P-value		
	Control group	Anaemic group	Control group	Anaemic group	Control group	Anaemic group	Diet	Anaemia	Diet × Anaemia
Food intake (g rat^{-1} d^{-1})	17.2±0.5a	19.0±0.3Ad	14.7±0.6b	15.3±0.6B	14.0±0.5b	13.8±0.2C	0.001	0.01	NS
Fe intake (μg rat^{-1} d^{-1})	792.8±23.4a	874.9±13.1Ad	656.0±27.2b	685.4±25.4B	614.7±22.4b	605.9±9.7C	0.001	0.05	NS
Faecal Fe (μg rat^{-1} d^{-1})	577.4±35.0a	510.7±13.6A	461.5±25.9b	381.2±13.1Bd	387.6±17.3b	286.7± 6.1Cd	0.001	0.001	NS
Absorbed Fe (μg rat^{-1} d^{-1})	215.4±21.4a	364.2±12.9Ad	194.4±13.4a	304.3±19.0Bd	227.0±10.7a	319.2±8.2ABd	NS	0.001	NS
ADC (%)	27.5±2.9a	41.6±1.3Ad	29.9±2.0a	44.0±1.9Ad	37.0±1.3b	52.6±0.9Bd	0.001	0.001	NS
Urinary Fe (μg rat^{-1} d^{-1})	5.96±0.69a	8.91±1.25A	4.51±0.55a	4.30±0.50B	4.62±0.27a	6.81±1.09AB	0.001	0.01	NS
Fe balance (μg rat^{-1} d^{-1})	209.4±21.7a	355.3±12.8Ad	189.9±13.5a	299.9±18.8Bd	222.4±10.7a	312.4±8.01ABd	0.05	0.001	NS
R/I (%)	26.7±2.9a	40.6±1.4Ad	29.2±2.0a	43.4±1.9Ad	36.2±1.3b	51.5±0.8Bd	0.001	0.001	NS

[a]Mean value±SEM for nine to ten rats per group. Mean values among groups with different letters differ ($P<0.05$) (a, b for control animals; A, B, C for anaemic animals; d for control vs. anaemic animals).
[b]ANOVA P-values are less than the probability listed; for NS, $P>0.05$.

Comparison of diets G and S showed that among the control animals the Fe content was similar for all the organs studied, except in the femur, where Fe content was greater for diet G ($P<0.001$), while in the anaemic rats fed with diet G, the Fe deposit was lower in the liver and the sternum ($P<0.001$), higher in the femur ($P<0.001$) and similar in the spleen. However, comparison of diets C and S showed that the Fe content in the control animals was similar in the spleen and femur and lower in the liver and sternum ($P<0.001$) (Table 4).

3.3. Haematological studies

In the control rats, all the haematological parameters studied, namely Hb concentration, serum Fe, RBC, PCV and platelets were within normal limits for this species at the start and end of the experimental period. After consuming the low-Fe diet for 40 d, the rats were anaemic, with a mean blood Hb concentration of 82.0 g L^{-1}. PCV and serum Fe were low, consistent with Fe-deficiency-induced anaemia in rats. The level of Hb increased after Fe-normal diets were provided (Tables 5 and 6).

The percentage HRE (Hb-Fe gain/Fe intake) was affected by the diet and by anaemia (two-way ANOVA, $P<0.001$). For the three diets assayed, HRE in Fe-deficient rats was higher than among controls ($P<0.001$). The percentage HRE was higher in both the control and the anaemic rats that consumed diet G than in those given diets S or C, which were similar among them (G:S, in controls $P<0.01$ and in anaemic $P<0.001$; G:C, in controls $P<0.05$ and anaemic $P<0.025$) (HRE: G > S = C) (Table 5).

Significant effects of diet and anaemia were observed on serum levels of Fe (two-way ANOVA, $P<0.001$). In addition, an interaction effect between diet and anaemia was observed (two-way ANOVA, $P<0.025$). Serum Fe decreased markedly among the anaemic rats given diet C ($P<0.001$) or diet S ($P<0.025$) in comparison with the relevant controls; however, the GM diet did not produce differences between the groups. Among the control rats, serum levels of Fe were similar for all the diets assayed. Nevertheless, for the anaemic rats, serum Fe was significantly higher among those given diet G ($P<0.001$) or diet S ($P<0.01$) than in those given diet C (Serum Fe: G = S > C) (Table 6).

Two-way ANOVA showed that RBC and PCV were affected by the anaemia before and after consuming the diets tested ($P<0.001$). Moreover, RBC final was influenced by the diet ($P<0.001$) and an interaction effect between diet and anaemia was observed ($P<0.001$) in PCV. Among Fe-deficient rats, RBC and PCV were lower than in the control rats for the three diets studied at the start of the EP ($P<0.001$). The Fe-deficient rats showed complete recovery of RBC and PCV after consuming the different diets (S, C and G), especially in rats fed diet S versus those given diet C ($P<0.001$ for RBC) (Table 6).

Significant effects of anaemia were deduced from the platelet count at the start and end of the EP (two-way

Table 4
Iron concentrations in several organs in control and iron-deficient rats fed different diets[a]

	Standard diet		Cow milk diet		Goat milk diet		Two-way ANOVA[b] P-value		
	Control group	Anaemic group	Control group	Anaemic group	Control group	Anaemic group	Diet	Anaemia	Diet × Anaemia
Spleen (mg kg^{-1} dry wt)	2397.3±180.3a	826.6±100.8Ad	2483.3±124.5a	405.0±54.3Bd	2810.0±277.4a	718.8±68.5Ad	NS	0.001	NS
Liver (mg kg^{-1} dry wt)	816.5±49.1a	558.0±45.1Ad	655.0±41.3b	338.9±19.7Bd	692.8±32.9ab	367.8±38.6Bd	0.001	0.001	NS
Sternum (mg kg^{-1} dry wt)	146.0±5.4a	158.7 ±11.3Ad	109.9±2.6b	78.9±2.5Bd	148.2±4.3a	111.3±2.7Cd	0.001	0.001	0.001
Femur (mg kg^{-1} dry wt)	112.8±6.5a	95.6±1.9Ad	117.7±6.2a	80.4±2.9Bd	174.2±6.6b	120.9±8.1Cd	0.001	0.001	0.01

[a]Mean value±SEM for nine to ten rats per group. Mean values among groups with different letters differ ($P<0.05$) (a, b for control animals; A, B, C for anaemic animals; d for control vs. anaemic animals).
[b]ANOVA P-values are less than the probability listed; for NS, $P>0.05$.

Table 5
Haemoglobin regeneration efficiency in control and iron-deficient rats fed different diets[a]

	Standard diet		Cow milk diet		Goat milk diet		Two-way ANOVA[b] *P*-value		
	Control group	Anaemic group	Control group	Anaemic group	Control group	Anaemic group	Diet	Anaemia	Diet × Anaemia
Initial body weight (g)	265.7±8.1a	237.8±7.0Ad	280.2±4.8a	258.8±3.6Ad	269.4±11.4a	264.3±11.5A	NS	0.01	NS
Final body weight (g)	292.4±7.9a	271.4±7.6Ad	304.9±4.7a	284.1±4.1Ad	286.0±12.3a	278.8±11.8A	NS	0.025	NS
Initial Hb concentration ($g\,L^{-1}$)	128.0±2.0a	89.0±2.0Ad	142.0±2.0b	74.0±3.0Bd	130.0±3.0a	82.0±1.0Ad	NS	0.001	0.001
Final Hb concentration ($g\,L^{-1}$)	135.0±2.0a	117.0±2.0Ad	146.0±2.0b	102.0±5.0Bd	142.0±2.0b	113.0±2.0Ad	NS	0.001	0.001
HRE (%)	15.7±0.9a	26.9±1.1Ad	16.7±1.3a	29.0±1.2Ad	21.4±1.5b	35.8±1.6Bd	0.001	0.001	NS

[a]Mean value±SEM for nine to ten rats per group. Mean values among groups with different letters differ ($P<0.05$) (a, b for control animals; A, B for anaemic animals; d for control vs. anaemic animals).
[b]ANOVA *P*-values are less than the probability listed; for NS, $P>0.05$.

Table 6
Haematological parameters in control and iron-deficient rats fed different diets[a]

	Standard diet		Cow milk diet		Goat milk diet		Two-way ANOVA[b] *P*-value		
	Control group	Ànaemic group	Control group	Anaemic group	Control group	Anaemic group	Diet	Anaemia	Diet × Anaemia
Serum iron ($\mu g\,L^{-1}$)	1819±136a	1431±103Ad	1524±91a	884±14Bd	1795±84a	1731±90A	0.001	0.001	0.025
Initial RBC ($10^{12}\,L^{-1}$)	7.16±0.18a	5.29±0.13Ad	7.08±0.18a	5.79±0.31Ad	7.44±0.17a	5.73±0.29Ad	NS	0.001	NS
Final RBC ($10^{12}\,L^{-1}$)	7.38±20a	7.81±0.23AB	6.90±0.21a	7.25±0.08A	7.44±0.17a	8.24±0.19Bd	0.001	0.001	NS
Initial PCV (%)	41±4.1a	26±1.0Ad	40±1.1a	25±1.2Ad	39±0.9a	24±0.9Ad	NS	0.001	0.001
Final PCV (%)	44±1.5a	42±1.0A	42±1.5a	40±1.0A	43±1.1a	42±1.4A	NS	0.001	0.001
Initial platelets (%)	686±20a	1492±110Ad	713±18a	1755±99A	722±23a	1498±146Ad	NS	0.001	NS
Final platelets (%)	666±22ab	859±44Ad	589±30a	918±55Ad	713±31b	886±50Ad	NS	0.001	NS

[a]Mean value±SEM for nine to ten rats per group. Mean values among groups with different letters differ ($P<0.05$) (a,b for control animals; A,B for anaemic animals; d for control vs. anaemic animals).
[b]ANOVA *P*-values are less than the probability listed; for NS, $P>0.05$.

ANOVA, $P<0.001$). In the Fe-deficient rats, platelets were significantly higher than in the control rats, for the three diets assayed at the beginning and at end of the EP ($P<0.001$, except $P<0.01$ for GM group at the end). After consuming the different diets, the platelet count in the anaemic rats was similar to the respective controls, especially for diet G (Table 6).

4. Discussion

The lower food intake of rats consuming the milk-based diets in comparison with diet S, observed previously by us for the diet G (Alférez et al. 2001; López-Aliaga, Alférez, Barrionuevo, Nestares, Sanz-Sampelayo, & Campos, 2003), could be due to the different sensory characteristics arising from the lyophilates used in their preparations, especially in the case of diet G.

The digestive utilization of Fe was greater in anaemic rats than in their controls, regardless of the type of diet consumed (Table 3). In fact, the negative correlation found between Fe intake and ADC was less pronounced for Fe-deficient rats ($r = 0.459$ and $P = 0.011$) than their controls ($r = 0.518$ and $P = 0.004$). This reveals that there is a greater avidity for Fe among anaemic rats, which might be because ferropenic anaemia increases the number of membrane receptors affected by non-heme Fe absorption and thus increases Fe absorption (Forellat, Gautier, & Fernandez, 2000). The increase in the ADC percentage of Fe in deficient rats is similar to that reported by Pallarés et al. (1993).

Fe balance and the R/I ratio were also greater in anaemic rats than in their controls for the three diets studied. The type of diet supplied influences the digestive use of Fe; both the groups of rats given diet G had a higher ADC and Fe retention, especially the Fe-deficient rats with respect to those consuming diet C. Studies of nutritive components of food have shown that milk-derived Ca strongly inhibits the absorption of heme and non-heme Fe (Hallberg, Brune, Erlandsson, Sandberg, & Rossander-Hulten, 1991). However, a mechanism that produces a direct effect of Ca on Fe absorption has yet to be identified. There may be a degree of competitive inhibition between Ca and Fe in the final transport from the intestinal mucosa to the plasma, which is valid for both heme and non-heme Fe (Hallberg et al., 1991).

The greater utilization of Fe with diet G could be due to various nutritional factors: the protein of this milk was more soluble and contained a higher proportion of other

soluble proteins (β-lactoglobulin, α-lactoalbumin and serum albumin) (Boza & Sanz-Sampelayo, 1997). This protein offered by diet G, due to its greater solubility and animal origin (Sharp, Tandy, & Srai, 2003), could favour the absorption of Fe.

The fat quality is different in the three diets; GM fat is richer in medium chain triglycerides (MCT) than the fat obtained from CM (Table 1). The MCTs in the diet are rapidly absorbed and metabolized to obtain energy (García-Unciti, 1996) and so could increase the synthesis of carrier proteins and thus the absorption of Fe.

There are some dietary components capable of reducing Fe (III) to Fe (II), including ascorbic acid (Wienk, Marx, & Beynen, 1999), and amino acids such as cysteine (Glahn & Van Campen, 1997). It is well-known that vitamin C is an important promoter of Fe absorption, as it forms a chelate with this mineral, which remains soluble at a higher pH within the small intestine (Czajka-Narins, 1996). Moreover, GM contains a greater quantity of cysteine (Table 1), an amino acid that is influential in improving Fe absorption (Van Campen, 1973).

Another factor to be taken into consideration is that GM has more than twice the vitamin A content of CM (Table 1). It has been suggested that vitamin A influences Fe metabolism, but this mechanism has not yet been fully elucidated. It is unlikely that vitamin A could interfere directly with the intestinal absorption of Fe, but it may mobilize available Fe stores and use them to form Hb (Bloem, 1995). Another possibility is the reduction of transferrin levels by vitamin A deficiency, which would then reduce Fe transportation (Bloem et al., 1989).

In addition, GM has higher vitamin D content than CM (Table 1). The positive role of vitamin D as a promoter of the active component in the absorptive process of Fe has been reported previously; this vitamin may directly favour Fe absorption by increasing the concentration of Fe-binding protein (mobilferrin) (Hartiti et al., 1995).

Among the body organs, the spleen and liver usually have the highest Fe concentration (Underwood, 1977) followed by other organs such as the sternum and the femur.

Fe concentrations decreased in all the tissues of anaemic rats that were analysed, compared with their controls, regardless of the diet supplied. Similar findings have been reported by other authors (Katsuhiko, Mieko, & Yoshinori, 1991; Shiguang, Clive, & Anton, 1994). Therefore, the marked decreases in Fe concentration in the liver and the spleen are suggestive of the depletion of Fe storage in anaemic rats.

In both the control and the anaemic animals, the lower Fe content in the spleen, liver, sternum and femur in the rats given diet C, in comparison with those consuming diet G, may be due to fact that Ca derived from CM interferes with the absorption of Fe in the diet (Hallberg et al., 1991). When the rats consumed diet G, the Fe stores were more replete, perhaps because this type of milk decreases Ca–Fe interference (López-Aliaga et al., 2000). This is in agreement with the better Fe R/I ratio achieved with the GM diet in present study.

After feeding the Fe-deficient rats for 14 d with the three experimental diets, there was recovery of anaemia, as assessed by higher serum levels of Fe, RBC and PCV levels and a lower platelet count, especially when the rats were fed with diet G. Our haematological data are in agreement with data from other studies (Pallarés et al., 1993; Wienk, Marx, Lemmens, Brink, Van der Meer, & Beynen, 1996).

The HRE percentage among the control groups was similar to that reported by other authors (Matsumoto, Mori, Doi, Kishida, & Ebihara, 2003). The greater digestive utilization of Fe by anaemic rats was reflected in the HRE, which increased markedly for the three diets (Table 6). These values were similar to those obtained in previous studies (Benito, House, & Miller, 1998; Pallarés et al., 1993). The HRE percentage was higher in both groups of animals, especially for the anaemic rats given diet G compared with diets S and C, which may be due, in part, to the higher nutritive utilization of Fe among the animals given diet G.

It is usually accepted that the sequence of events during oral treatment of Fe-deficiency anaemia is, first, the normalization of the Hb value, followed by restoration of storage Fe (Bothwell & Finch, 1962). Under our experimental conditions, there was an inverse correlation between Hb-Fe gain and spleen stores ($r = 0.620$, $P = 0.001$) and liver stores ($r = 0.577$, $P = 0.001$). This means that although anaemic rats responded rapidly to Fe dietary repletion with respect to haematology and storage of non-heme organ Fe, the anaemic rats showed a significantly lower Fe concentration than did their controls. The present results indicate, therefore, an incomplete restoration of Fe stores within the time frame (14 d) of this experiment.

5. Conclusions

Under our experimental conditions, dietary GM improves Fe bioavailability, increasing its digestive and metabolic utilization and Fe deposit in target organs and favouring the recovery of normal haematological parameters after nutritional ferropenic anaemia. The beneficial effects of GM with respect to nutritional ferropenic anaemia, as described in the present study, suggest that the inclusion of GM in the diet among the population at large may help in the prevention of the development of Fe-deficiency anaemia, but further investigation in humans is needed.

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