Effects of goats' or cows' milks on nutritive utilization of calcium and phosphorus in rats with intestinal resection

Margarita S. Campos*, Inmaculada López-Aliaga, María J. M. Alférez, Teresa Nestares and Mercedes Barrionuevo

Department of Physiology, Institute of Nutrition and Food Technology, University of Granada, E-18071 Granada, Spain

We analysed the effects of goats' milk (GM) on the nutritive utilization of Ca and P in rats with resection of 50 % distal small intestine in comparison with cows' milk (CM) and a standard non-milk diet. The three test diets contained 200 g protein and 100 g fat/kg. The apparent digestibility coefficient (ADC) of Ca and P were considerably higher in the two groups of rats given the GM diet than those given the other two diets. Ca and P retention did not decrease by effect of intestinal resection with GM diet. In both groups of animals, serum Ca and P levels and ionic Ca were higher in the case of the GM diet than the other two diets, whereas the parathyroid hormone levels were lower. Ca content in femur, sternum and *longissimus dorsi* muscle was higher in rats given the GM diet. P content in femur and sternum was higher among the two groups of rats given a milk-based diet (GM or CM), especially with GM diet. The GM diet has beneficial effects on nutritive utilization of Ca and P in control rats and those with resection of the distal small intestine.

Calcium: Phosphorus: Goats' milk: Cows' milk: Intestinal resection

In an earlier study we showed that the distal resection of the small intestine provokes adverse effects on the homeostasis of Ca and P in rats (Barrionuevo *et al.* 1989; Campos *et al.* 1989). The modification of lipids in the diet using a mixture of equal parts of medium-chain triacylglycerol, olive oil and sunflower oil raises Ca and P absorption, preferentially by active transport (López Aliaga *et al.* 1994; Alférez *et al.* 1996), both in transected (control) animals and in those with resection of 50 % distal small intestine.

One of the main reasons why milk is considered a food of exceptional quality is due to the minerals it provides, and especially Ca and P, in optimum proportions for absorption (Ca:P 1·0–1·5). While both milk and milk products are an important source of Ca, goats' milk (GM) is especially so. As remarked by Moreno (1995), the consumption of a suitable quantity of Ca, in proportion to that of P, would be difficult without the inclusion in the diet of a considerable amount of milk or milk-based products. GM is rich in medium-chain triacylglycerol compared with human milk and cows' milk (CM) (Haenlein, 1996), a factor that is important in the nutritive utilization of Ca and P (López Aliaga *et al.* 1994; Alférez *et al.* 1996).

Taking these considerations into account, we studied the effects of GM on the digestive and metabolic utilization of Ca and P and the mineral content in various organs of transected (control) and resected rats (50 % distal small intestine). A comparative study was made with a CM diet and a non-milk diet recommended by the American Institute of Nutrition (1977).

Materials and methods

Animals

The study carried out on sixty-nine animals (white male rats, Ratus novergicus, Wistar albino breed), with an initial body weight of 177 (SE 3) g, obtained from the University of Granada Laboratory Animal Service. After surgery, both the transected (control) and resected animals were housed in individual, ventilated, thermoregulated (22 ± 2°C) cages with a 12 h light–dark period. Food and mineral-free water were available *ad libitum* to all rats.

Assurance of compliance

All experiments and surgical procedures using rats conformed to the guidelines and legal requirements established in the UK for the proper care and use of laboratory animals.

Diets

The diets, mineral and vitamin supplements were prepared according to the recommendations of the American

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Institute of Nutrition (1977), except that the level of fat in the diets was 100 rather than 50 g/kg (Table 1). The standard non-milk diet was prepared using olive oil as the source of fat (100 g/kg) and casein as the protein source (200 g/kg). The milk-based diets were created with lyophilized CM or GM respectively. These were analysed to determine the fat (CM 352·3, GM 436·3 g/kg), protein (CM 239·2, GM 252·7 g/kg) and lactose (CM 355·5, GM 311·0 g/kg) contents and mineral compositions (mg/kg lyophilized milk): CM Ca 10315, P 7313, Mg 763, Fe 6·1, Cu 1·1, Zn 37·2; GM Ca 12152, P 8433, Mg 825, Fe 11·3, Cu 4·2, Zn 41·5. The necessary quantities of lyophilized CM or GM were taken to obtain a diet with a fat content of 100 g/kg. To obtain the protein content of 200 g/kg (as recommended by the American Institute of Nutrition (1977)) the diet was supplemented with casein (125·3 g casein/kg CM diet and 140·5 g casein/kg GM diet), as the protein provided by the lyophilate used for the milk-based diets was insufficient.

Table 1. Composition of the experimental diets

Component	g/kg diet (dry weight)
Diet S (standard non-milk)	
Protein (casein)	209
DL-Methionine	3
Fat (olive oil)*	112
Fibre (micronized cellulose)	50
Mineral supplement†	36
Vitamin supplement†	10
Choline chloride	2
Wheat starch	156
Sucrose	450
Energy (kJ/kg diet)	17890
Diet CM	
Protein (casein+CM protein)	190
DL-Methionine	3
Fat (CM)‡	98
Fibre (micronized cellulose)	40
Mineral specific supplement§	35
Vitamin specific supplement§	10
Choline chloride	2
Wheat starch	152
Sucrose	487
Energy (kJ/kg diet)	17598
Diet GM	
Protein (casein+GM protein)	194
DL-Methionine	3
Fat (GM)‖	92
Fibre (micronized cellulose)	46
Mineral specific supplement§	36
Vitamin specific supplement§	10
Choline chloride	2
Wheat starch	153
Sucrose	485
Energy (kJ/kg diet)	17422

CM, cows' milk; GM, goats' milk.
* Contains a 0·0 g medium-chain triacylglycerol/kg.
† Mineral and vitamin supplements were prepared according to the recommendations of the American Institute of Nutrition (1977).
‡ Contains 20·6 g medium-chain triacylglycerol/kg.
§ Mineral and vitamin specific supplements were formulated taking into account the mineral content of the lyophilized milks supplied in order to meet the mineral-content recommendations of the American Institute of Nutrition (1977).
‖ Contains 33·2 g medium-chain triacylglycerol/kg.

The mineral supplements were prepared according to American Institute of Nutrition (1977) recommendations for the standard non-milk diet and to our own specifications for the milk-based diets. These specific supplements were formulated taking into account the mineral content of the lyophilized milks supplied to the rats in order to meet the mineral content recommendations of the American Institute of Nutrition (1977; 5200 mg Ca and 4000 mg P/kg diet).

The lactose content of the milk diets was subtracted from the total carbohydrate content of the standard diet and wheat starch and sucrose was added corresponding to the difference (Table 1).

Resection and transection procedures

The method described by Hartiti *et al.* (1994) was used to carry out the resection of 50 % of the distal small intestine in rats. Animals in which the intestine was transected were treated identically except that the small intestine was only divided and reanastomosed at the mid-small intestine, without exclusion of any part of intestine. These transected rats were the control groups, because they maintained the whole intestine and all the blood supply.

Experimental design

Six experimental groups were formed: (1) group T-S, transected (control) rats, standard non-milk diet (*n* 11); (2) group R-S, resected rats, standard non-milk diet (*n* 13); (3) group T-CM, transected (control) rats, CM diet (*n* 10); (4) group R-CM, resected rats, CM diet (*n* 11); (5) group T-GM, transected (control) rats, GM diet (*n* 14); (6) group R-GM, resected rats, GM diet (*n* 10).

All animals were fed up to the time of surgery, and were given access to water containing 50 g glucose/l for 24 h after surgery. Thereafter, a period of 30 d was allowed for adaptation to the diet, during which feed and mineral-free water were available *ad libitum* to all animals. Beginning 30 d after surgery, food intake (the amount of food consumed per d by each rat determined by weighing the amounts of diet given, refused and spilled) was measured and urine and faeces were collected each d for a period of 7 d (Thomas & Mitchell, 1923). The urine for the 7 d of the experimental period was collected on HCl (5 ml/l), filtered (Whatman filter paper no. 42; Whatman, Maidstone, Kent, UK) and diluted. The faeces for the 7 d experimental period were dried, weighed and homogenized. Body weight was recorded at the beginning and end of the experimental period. Throughout the experimental period all rats had access to mineral-free water. At the end of this period, all animals were fasted for 24 h and killed after intraperitoneal anaesthesia with sodium pentobarbital (50 mg/kg body weight) and totally bled by cannulation of the abdominal aorta. The entire volume of blood was centrifuged at 3500 rpm for 12 min to separate the serum, which was frozen at −30°C until biochemical analysis. The femur, sternum, *longissimus dorsi* muscle, liver, spleen, kidneys, heart, brain and testes were dissected, frozen at −20°C, freeze-dried, homogenized and kept dry until analysed.

Biological indices

The apparent digestibility coefficient (ADC) and balance were calculated according to the following formulas:

$$ADC \ (\%) = (absorbed/intake) \times 100,$$

where

$$nutrient \ absorption = intake - faecal \ excretion,$$

and

$$balance = intake - (faecal + urinary \ excretion).$$

Analytical techniques

Dry matter. Water content in the diet, faeces, femur, sternum, *longissimus dorsi* muscle, liver, spleen, kidney, heart, brain and testis was determined by drying the material at $105 \pm 2°C$ until the weight remained constant (about 48 h).

Ash. A sample of the resulting sample (diet, faeces and liver; 1–2 g) or the entire sample (femur, sternum, *longissimus dorsi* muscle, spleen, kidney, heart, brain, testis) was oven-ashed at 450°C. The residue obtained was weighed and then diluted in 5 M-HCl to which mineral-free water was added to a predetermined volume for subsequent analysis.

Mineral determination. The concentration of Ca in the diet, faeces and the different organs were determined by atomic absorption spectrophotometry (Perkin-Elmer 1100 B; Perkin-Elmer, Shelton, CT, USA) and compared with a series of standard values. The concentration of P in the diet, faeces, serum and the different organs was analysed by visible spectrophotometry (Perkin-Elmer UV/vis spectrometer lambda 16) using the Fiske–Subbarow technique (Fiske & Subbarow, 1925).

Serum calcium concentrations. The serum concentrations of Ca were determined by colorimetry, using the method of Sarkar & Chauvan (1967).

Serum ionized calcium. Ionic Ca was measured with an autoanalyser using a NOVA 7 selective electrode (Nova Biomedical, Waltham, MA, USA).

Parathyroid hormone. Parathyroid hormone was determined with a radioimmunoassay for the C-terminal/half molecule of the hormone (Nichols Institute, San Juan Capistrano, CA, USA) measured with a Packard counter (Packard, Meriden, CT, USA).

Quality control

Given the importance of accurate determination of the various variables studied, the measurements of these were subjected to a quality control procedure. This consisted of analysing a skimmed milk powder (certified reference material CRM 063R; Community Bureau of Reference, Brussels, Belgium), which yielded a mean Ca value 13·89 (SEM 0·10) and a P value 10·99 (SEM 0·12) mg/g (*n* 5 determinations) (certified values: Ca 13·49 (SEM 0·10) and P 11·10 (SEM 0·13) mg/g).

Statistical analyses

We calculated the mean values with their standard errors for each variable studied. The data were analysed statistically by two-way ANOVA using a model with two main effects (animal group and type of diet) (SPSS, version 9.0.1, 2001; SPSS Inc., Chicago, IL, USA). Values of $P < 0.05$ were considered significant.

Results

Apparent digestibility coefficient and balance of calcium and phosphorus

The ADC for Ca and P were significantly reduced in the resected animals for the three diets studied ($P < 0.05$). The ADC of Ca and P were considerably higher in the two groups of rats given the GM diet than those given the other two diets, non-milk standard and CM (standard non-milk diet $P < 0.01$ for Ca and $P < 0.001$ for P in transected and resected rats; CM $P < 0.001$ for Ca and P in transected and resected rats respectively). The ADC for Ca and P in both animals groups fed the standard non-milk diet and CM diet did not differ significantly (ADC of Ca and P GM > standard non-milk=CM) (Tables 2 and 3). The Ca and P balance did not vary between the transected (control) and the resected animals fed with GM diet and were reduced in resected rats fed with CM diet or with standard non-milk diet ($P < 0.05$) for Ca and P. In transected (control) and resected animals, the Ca and P retention were greater when the GM diet was consumed than when the standard non-milk diet was given (transected rats $P < 0.05$ for Ca and $P < 0.01$ for P, resected rats $P < 0.01$ for Ca and $P < 0.001$ for P) (Tables 2 and 3). In transected rats, the retention of Ca was higher with the CM diet than the standard non-milk diet ($P < 0.05$). To compare both types of milk, it is evident that only the P retention was higher in resected rats fed with GM diet in relation to resected rats fed with CM diet ($P < 0.01$) (Tables 2 and 3).

Serum concentrations of calcium (total and ionic), phosphorus and parathyroid hormone

The concentrations of total Ca and P in serum were not affected by the intestinal resection, for any of the three diets studied (Table 4). In the case of Ca, among the transected (control) and resected rats, the serum concentrations were higher when a milk diet (GM or CM) was consumed than when the standard non-milk diet was given ($P < 0.001$); in particular, the serum Ca was higher when the animals consumed the GM diet in comparison with the CM diet ($P < 0.001$) (Table 4). However, serum ionized Ca was affected by intestinal resection for the three diets tested (standard diet $P < 0.05$, CM diet $P < 0.01$, GM diet $P < 0.001$), whereas the effect of different type of diets on ionic Ca shows a similar pattern to total serum Ca, thus the serum ionized Ca was higher when a milk diet, specially GM, diet was consumed ($P < 0.001$) (Table 4).

In transected (control) and resected rats, the concentrations of P in serum were higher when the GM diet was consumed than either the CM diet ($P < 0.001$) or the standard non-milk diet ($P < 0.001$). Standard non-milk and CM diets resulted in similar concentrations of P in serum in both animals groups (Table 4).

Table 2. Digestive and metabolic utilization of calcium in transected and resected rats fed on standard non-milk or milk diets (cows' (CM) or goats' (GM))§

(Mean values with their standard errors)

Group	n	Ca intake (mg/rat per d) Mean	SE	Faecal Ca (mg/rat per d) Mean	SE	Absorbed Ca (mg/rat per d) Mean	SE	ADC (%) Mean	SE	Urinary Ca (mg/rat per d) Mean	SE	Ca balance (mg/rat per d) Mean	SE
T-S	11	87·1	2·8	59·2	2·5	28·0	1·6	32·2	1·5	1·9	0·1	26·1	1·6
R-S	13	89·1	3·1	65·5	2·9	23·6	1·4	26·5*	1·7	1·7	0·2	21·9*	1·4
T-CM	10	105·4†††	2·5	73·0†††	3·1	32·4	2·2	30·8	2·0	1·9†††	0·1	30·5†	1·2
R-CM	11	112·8†††	3·0	83·6*†††	2·2	29·3††	1·5	25·9*	1·0	3·5*††	0·4	25·7*	1·4
T-GM	14	83·6‡‡‡	1·7	51·3††‡‡‡	0·9	32·3	1·5	38·4††‡‡‡	1·3	0·7†††‡‡	0·1	31·6†	1·5
R-GM	10	86·8‡‡‡	3·2	57·2††‡‡‡	2·8	29·6††	1·5	34·1*††‡‡	1·6	0·8‡‡	0·3	28·7††	1·3

ADC, apparent digestibility coefficient; T-S, transected rats fed on the standard non-milk diet; R-S, resected rats fed on the standard non-milk diet; T-CM, transected rats fed on the CM diet; R-CM, resected rats fed on the CM diet; T-GM, transected rats fed on the GM diet; R-GM, resected rats fed on the GM diet.
Mean values were significantly different from those of the transected groups: *$P<0.05$.
Mean values were significantly different from those corresponding standard non-milk group: †$P<0.05$. ††$P<0.01$. †††$P<0.001$.
Mean values were significantly different from those of the coresponding CM group: ‡‡$P<0.01$. ‡‡‡$P<0.001$.
§ For details of diets and procedures see Table 1 and pp. 61–62.

Table 3. Digestive and metabolic utilization of phosphorus in transected and resected rats fed on standard non-milk or milk diets (cows' (CM) or goats' (GM))§

(Mean values with their standard errors)

Group	n	P intake (mg/rat per d) Mean	SE	Faecal P (mg/rat per d) Mean	SE	Absorbed P (mg/rat per d) Mean	SE	ADC (%) Mean	SE	Urinary P (mg/rat per d) Mean	SE	P balance (mg/rat per d) Mean	SE
T-S	11	71·7	2·3	31·9	1·7	39·8	1·5	55·6	1·4	25·0	1·4	14·8	1·7
R-S	13	73·3	2·5	35·9	1·3	36·7	1·7	50·0*	1·4	27·2	1·3	9·5*	1·2
T-CM	10	81·7†††	2·0	36·4†	1·5	45·3††	1·6	55·5	1·5	26·1	1·4	19·2	2·2
R-CM	11	85·6††	1·4	42·4**††	1·4	43·3††	1·7	50·6*	1·6	30·4	1·8	12·9*	1·6
T-GM	14	68·0‡‡‡	1·4	22·9†††‡‡‡	0·9	45·1††	1·0	66·5†††‡‡‡	1·1	23·1	1·1	22·0††	1·2
R-GM	10	70·5‡‡‡	1·6	26·5†††‡‡‡	1·9	44·0††	1·4	62·4*†††‡‡	1·4	24·0‡‡	1·3	20·0†††‡‡	1·8

ADC, apparent digestibility coefficient; T-S, transected rats fed on the standard non-milk diet; R-S, resected rats fed on the standard non-milk diet; T-CM, transected rats fed on the CM diet; R-CM, resected rats fed on the CM diet; T-GM, transected rats fed on the GM diet; R-GM, resected rats fed on the GM diet.
Mean values were significantly different from those of the transected groups: *$P<0.05$, **$P<0.01$.
Mean values were significantly different from those corresponding standard non-milk group: †$P<0.05$. ††$P<0.01$. †††$P<0.001$.
Mean values were significantly different from those of the coresponding CM group: ‡‡$P<0.01$. ‡‡‡$P<0.001$.
§ For details of diets and procedures see Table 1 and pp. 61–62.

Table 4. Serum concentrations of calcium (total and ionic), phosphorus and parathyroid hormone in transected and resected rats fed on standard non-milk or milk diets (cows' (CM) or goats' (GM))§

(Mean values with their standard errors)

	T-S (n 11) Mean	SE	R-S (n 13) Mean	SE	T-CM (n 10) Mean	SE	R-CM (n 11) Mean	SE	T-GM (n 14) Mean	SE	R-GM (n 10) Mean	SE
Serum Ca (mg/l)	100·1	0·7	98·2	0·7	107·3†††	0·6	106·1†††	0·8	114·3†††‡‡‡	0·8	112·2†††‡‡‡	0·9
Serum ionized Ca (mg/l)	58·1	0·3	57·3*	0·4	61·4†††	0·4	59·3**†††	0·4	67·2†††‡‡‡	0·5	64·1***†††‡‡‡	0·4
Serum P (mg/l)	73·4	1·9	73·0	1·3	74·3	1·1	73·2	1·6	80·6†††‡‡‡	0·9	80·2†††‡‡‡	1·4
Parathyroid hormone (ng/l)	121·3	3·4	124·4	4·3	109·2†	3·3	110·9†	4·8	98·2††‡	2·7	101·1†††	2·4

T-S, transected rats fed on the standard non-milk diet; R-S, resected rats fed on the standard non-milk diet; T-CM, transected rats fed on the CM diet; R-CM, resected rats fed on the CM diet; T-GM, transected rats fed on the GM diet; R-GM, resected rats fed on the GM diet.
Mean values were significantly different from those of the transected groups: *$P<0.05$, **$P<0.01$, ***$P<0.001$.
Mean values were significantly different from those corresponding standard non-milk group: †$P<0.05$. ††$P<0.01$. †††$P<0.001$.
Mean values were significantly different from those of the coresponding CM group: ‡$P<0.05$. ‡‡‡$P<0.001$.
§ For details of diets and procedures see Table 1 and pp. 61–62.

The serum concentrations of parathyroid hormone were similar in transected and resected rats with the three types of diet studied. In relation to the type of diet, the diets based on milk and especially the GM diet, showed the lowest serum concentrations of parathyroid hormone in transected rats (GM $P<0.01$, CM $P<0.05$) and resected rats (GM $P<0.001$, CM $P<0.05$) with respect to the standard non-milk diet. To compare both types of milk, the GM caused lower parathyroid hormone concentrations with respect to the CM in transected animals ($P<0.05$) (Table 4).

Mineral content in different organs

In general, the deposit of Ca and P in the femur, sternum and in the *longissimus dorsi* muscle was very little affected at 37 d after the intestinal resection for the three types of diet studied, except in resected rats fed with CM diet for which the Ca content in femur was lower ($P<0.05$) (Tables 5 and 6). In the case of Ca, in transected (control) and resected rats, no differences were observed between the animals given the non-milk standard and the CM diets, except in femur that in transected animals the Ca content was higher with the CM diet than standard non-milk diet ($P<0.001$); the mineral deposit was greater, always, in the case of the GM diet ($P<0.001$) (Table 5). In relation to P, and in general, the deposit in femur and sternum was greater in both animal groups given the milk-based diets, especially with GM diet (for significance levels, see Table 6). In the muscle, there was a greater

quantity of P for both the transected (control) and the resected rats when the GM diet was consumed ($P<0.001$), followed by the standard non-milk diet, while the lowest amount corresponded to the CM diet ($P<0.01$) for transected and resected rats (Table 6).

With respect to the other organs studied (liver, spleen, kidney, heart, brain and testis), the Ca content/g dry weight is in the order of μg, well below the quantity found in the femur, the sternum and the muscle. In general, these levels were little affected by the type of diet consumed (Table 5).

In the case of P, there was a notably higher content in the other organs studied (liver, spleen, kidney, heart, brain and testis) of the transected (control) and resected animals given the GM diet with respect to those consuming the CM diet (almost 2-fold, $P<0.001$) and the standard diet (except for heart and testis in transected rats, $P<0.001$) (Table 6).

Table 5. Calcium concentration in several organs (mg or μg/g dry weight) in transected and resected rats fed on standard non-milk or milk diets (cows' (CM) or goats' (GM))§

(Mean values with their standard errors)

	T-S (n 11)		R-S (n 13)		T-CM (n 10)		R-CM (n 11)		T-GM (n 14)		R-GM (n 10)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Femur (mg)	233.8	0.8	227.7	4.5	241.8†††	1.0	235.3*	2.7	260.5†††‡‡‡	1.6	258.7†††‡‡‡	5.0
Sternum (mg)	133.3	2.9	128.6	1.5	140.5	3.1	134.3	3.0	157.8†††‡‡‡	2.7	154.6†††‡‡‡	4.3
Longissimus dorsi (mg)	5.8	0.2	5.5	0.3	5.8	0.3	5.8	0.3	7.1††‡‡	0.3	7.0††‡‡	0.2
Liver (μg)	258.9	21.2	217.8	22.6	274.2	19.8	171.1***	8.3	301.0	15.4	237.1**‡‡	14.4
Spleen (μg)	148.3	9.1	142.0	7.2	128.5†	4.5	117.8*†††	2.9	108.9†††‡	2.4	123.7**†	3.0
Kidney (μg)	441.2	13.2	439.7	8.4	442.4	14.0	440.8	7.7	456.5	3.5	478.3†‡	16.2
Heart (μg)	88.4	2.5	83.7	2.4	106.2†††	4.0	97.9††	3.3	79.7†‡‡‡	2.7	92.9*	5.3
Brain (μg)	592.9	48.3	491.6	37.3	430.1††	37.3	464.4	28.6	613.3‡‡‡	31.3	666.2†††‡‡‡	19.9
Testis (μg)	249.6	5.2	233.4*	5.6	386.8†††	12.3	375.8†††	11.7	277.7†‡‡‡	8.1	333.2**††‡	16.7

T-S, transected rats fed on the standard non-milk diet; R-S, resected rats fed on the standard non-milk diet; T-CM, transected rats fed on the CM diet; R-CM, resected rats fed on the CM diet; T-GM, transected rats fed on the GM diet; R-GM, resected rats fed on the GM diet.
Mean values were significantly different from those of the transected groups: *$P<0.05$, **$P<0.01$, ***$P<0.001$.
Mean values were significantly different from those corresponding standard non-milk group: †$P<0.05$, ††$P<0.01$, †††$P<0.001$.
Mean values were significantly different from those of the coresponding CM group: ‡$P<0.05$, ‡‡$P<0.01$, ‡‡‡$P<0.001$.
§ For details of diets and procedures, see Table 1 and pp. 61–62.

Table 6. Phosphorus concentration in several organs (mg/g dry weight) in transected and resected rats fed on standard non-milk or milk diets (cows' (CM) or goats' (GM))§

(Mean values with their standard errors)

	T-S (n 11)		R-S (n 13)		T-CM (n 10)		R-CM (n 11)		T-GM (n 14)		R-GM (n 10)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Femur	83.1	1.4	81.1	1.2	87.3	1.9	85.5†	0.9	89.5†	2.4	87.5†	2.8
Sternum	43.1	1.5	40.1	1.2	46.9†	0.8	46.4†††	0.9	49.3††	0.7	48.8†††	1.0
Longissimus dorsi	5.1	0.3	4.9	0.4	4.0††	0.2	3.7††	0.2	6.7†††‡‡‡	0.2	6.4†††‡‡‡	0.1
Liver	5.1	0.2	5.8*	0.2	3.2†††	0.1	3.6†††	0.2	6.4†††‡‡‡	0.1	7.0**†††‡‡‡	0.2
Spleen	7.5	0.2	7.1	0.2	5.2†††	0.3	4.8†††	0.1	10.6†††‡‡‡	0.3	10.7†††‡‡‡	0.5
Kidney	5.9	0.1	5.9	0.1	4.2††	0.5	4.5†††	0.1	11.3†††‡‡‡	0.4	10.8†††‡‡‡	0.3
Heart	6.9	0.1	5.6***	0.3	4.9†††	0.2	3.6***†††	0.2	8.2‡‡‡	0.2	7.4*†††‡‡‡	0.3
Brain	8.2	0.1	8.1	0.1	5.8†††	0.3	5.7†††	0.1	13.9†††‡‡‡	0.5	12.3*†††‡‡‡	0.5
Testis	7.6	0.3	5.4***	0.3	4.9†††	0.1	4.7	0.1	8.1‡‡‡	0.2	7.5*†††‡‡‡	0.3

T-S, transected rats fed on the standard non-milk diet; R-S, resected rats fed on the standard non-milk diet; T-CM, transected rats fed on the CM diet; R-CM, resected rats fed on the CM diet; T-GM, transected rats fed on the GM diet; R-GM, resected rats fed on the GM diet.
Mean values were significantly different from those of the transected groups: *$P<0.05$. **$P<0.01$, ***$P<0.001$.
Mean values were significantly different from those of the corresponding standard non-milk group: †$P<0.05$, ††$P<0.01$, †††$P<0.001$.
Mean values were significantly different from those of the coresponding CM group: ‡‡‡$P<0.001$.
§ For details of diets and procedures, see Table 1 and pp. 61–62.

Discussion

The ADC of Ca and P were reduced by the intestinal resection for the three diets tested. Thus, with the standard diet the ADC of Ca and P in resected rats is reduced by 17·7 and 10·1 % respectively, while in previous studies carried out by our research group using rats with the same type of intestinal resection and diets with the same Ca and P content, this reduction was more pronounced (60·8 % for Ca and 46·1 % for P) (Barrionuevo *et al.* 1989; Campos *et al.* 1989). This minor effect might be explained by the fact that the quantities of protein (200 g/kg) and fat (100 g/kg) in the three diets tested were practically double those used in the earlier study (120 g protein and 50 g fat/kg) (Barrionuevo *et al.* 1989; Campos *et al.* 1989). In other studies with rats, it has been shown that an increase in the level of protein in the diet produces a corresponding increase in the absorption of Ca (Wapnir, 1989; Pallarés *et al.* 1993; Campos *et al.* 1996) and P (Pallarés *et al.* 1993; Campos *et al.* 1996). Moreover, Alférez *et al.* (1996) found that in rats with resection of the distal small intestine the proximal colon undergoes an adaptative response, there being an increase in the absorption capacity per unit of length and weight of intestinal mucosa. Thus, an increase in the level of protein in the diet, a factor that favours the bioavailability of Ca, together with the greater absorption of this mineral due to the adaptative capacity of the large intestine could contribute to a greater ADC of Ca. This present study confirms recent findings in human subjects and rats, which show that not only the distal intestine but also the hindgut may play an important role in Ca absorption (Younes *et al.* 1993, 1996, 2001; Greger, 1999).

With respect to the ADC of P, casein, which is the principal protein in the three diets tested, contains about 7·2 g P/kg casein, and when the proportion of casein in the diet rises from 120 to 200 g/kg, which represents an increase of 80 g casein/kg diet, there is a corresponding increase in the levels of P (Reeves *et al.* 1993). This increase in the quantity of P in the intestinal lumen of resected rats could contribute to raise the absorption of P by difussion.

On the other hand, this is in accordance with a recent study by Alférez *et al.* (2001), where the absorption of fat in animals with intestinal resection rises when the lipid proportion in the diet is increased (from 50 to 100 g/kg), and approaches the levels found in intact animals.

The consumption of a GM diet, both by the transected (control) and the resected animals, increased the digestive utilization of Ca and P with respect to the other two diets (the non-milk standard and the CM diet). This might be due to the different protein and lipid quality of GM with respect to CM and the standard non-milk diet. GM contains a higher quantity of lysine (3·4 g/l) than does CM (2·8 g/l) (Souci *et al.* 1989). The beneficial effect of this basic amino acid seems to be related to the non-mediated transport of Ca, and could be due to neutralization of organic acids by lysine, because these acids may bind to Ca and impair its absorption (Wapnir, 1990).

Moreover, GM has a higher medium-chain triacylglycerol content than CM (Haenlein, 1996) and increased micellar fatty acids in the ingesta increase local mucosal blood flow and perhaps increase passive uptake of Ca and P.

On the other hand, the greater absorption of Ca and P in animals given the GM diet could be explained by the high vitamin D content of GM (2·5 μg/l) with respect to CM (0·63 μg/l) (Souci *et al.* 1989); this vitamin favours the absorption of Ca (Campos *et al.* 1989; Alférez *et al.* 1996) and P (Barrionuevo *et al.* 1989; López Aliaga *et al.* 1994).

The results of the digestive utilization of Ca and P are complemented by the results for the balance (retention) of these two minerals.

The retentions of Ca and P were greater among the transected (control) than the resected animals, when the diet was non-milk standard or based on CM. When it was based on GM, it is noteworthy that there were no differences between the two groups of animals in this respect. This demonstrates the beneficial effects of the GM diet, which increases the retention of Ca and P in animals with intestinal resection. The retentions of Ca and P were higher amongst both groups of animals when the diets were based on milk, especially with GM, which could be due to the higher quantity of vitamin D in this type of milk (Souci *et al.* 1989). Vitamin D is known to have a beneficial effect on the retention of Ca (Campos *et al.* 1989).

The lower nutritive utilization of Ca and P in resected animals is reflected in only a small decline in the content of total Ca and P in serum. This effect is more evident when the ionic Ca is determined, and both the intestinal resection and the type of diet affected these concentrations. Thus, with the three diets studied the ionized Ca concentration was lower in rats with intestinal resection, and the milk-based diet (GM or CM), especially the GM milk diet, led to higher concentrations of both total and ionized Ca in serum. Blood pH was unchanged by the effect of resection, so the differences found in ionized Ca in serum could be justified because total plasma protein concentrations are affected by the intestinal resection and were relatively proportional to the changes in serum ionized Ca (López Aliaga *et al.* 2003). The parathyroid hormone concentrations showed a relationship between ionic Ca and this hormone and it is evident that when the ionic Ca concentrations were higher in rats fed with the milk-based diet, especially with GM diet, the parathyroid hormone serum concentrations were decreased.

At 37 d after the surgical intervention, the content or deposit of Ca in the preferential target organs for Ca (femur, sternum and the *longissimus dorsi* muscle) (Arnaud & Sánchez, 1997) was virtually unchanged. However, the Ca content was higher in the muscle and bone (femur and sternum) among the rats given the GM diet than among those consuming the other two diets. Thus, GM could play an important role in preventing Ca deficiency. With reference to the Ca content in the other organs studied (liver, spleen, kidney, heart, brain, testis), these were within the normal ranges described in the literature for this species (Lisbona *et al.* 1999).

The GM diet had a positive effect on the deposit of P in the organs where the high consumption of ATP requires its presence (i.e. muscle, brain, kidney and spleen).

The consumption of a natural food such as GM increases the nutritive utilization and deposit of Ca and P in the target organs for these minerals, both among the transected (control) and resected animals. In general, the GM reduces the negative effect of intestinal resection on Ca and P metabolism and approaches the values obtained to the transected animals (controls). For these reasons, extrapolating the results obtained in rats to man, the consumption of GM is advisable in situations of malabsorption syndrome and among the population in general.

Acknowledgements

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