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PII: S0308-8146(98)00050-8

Food Chemistry, Vol. 63, No. 3, pp. 299–305, 1998

Printed in Great Britain
0308-8146/98 $19.00 + 0.00

Content and *in vitro* availability of Fe, Zn, Mg, Ca and P in homogenized fish-based weaning foods after bone addition

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(Received 3 November 1997; revised version received 26 January 1998; accepted 26 January 1998)

The aim of the present study was to compare the Fe, Zn, Mg, Ca and P contents and their *in vitro* availability in five fish-based infant weaning foods, as a function of the fish bone added with the muscle. The weaning foods studied were: sole without bone (S); hake without bone (H); sole with bone and hake without bone (SBH); sole with bone (SB); and hake with bone (HB). Low levels of Fe and Zn and low percentages of these dialysed minerals were found in all the weaning foods assayed. The incorporation of bone increased the Ca and P contents, particularly in HB weaning food ($92.8 \pm 0.44\,mg\,100\,g^{-1}$ for Ca and $274 \pm 1.93\,mg\,100\,g^{-1}$ for P). The amounts of Ca and P dialysed were also higher in the weaning foods with bone.

INTRODUCTION

Infancy is probably the period of life when nutritional demands are greatest, since weight triples and length doubles during the first year of life. Such rapid growth means that large amounts of minerals are required (Milner, 1990; Bueno, 1993). Providing these minerals is important, since an inadequate intake of certain minerals can produce diseases and lead to an abnormal development (Milner, 1990). Ca and P are essential for optimal bone mineralization (Cruz and Tsang, 1992), and children fed diets deficient in these minerals can develop rickets (Pettifor, 1991). However, not only is the quantity of Ca and P in the diet important, but also the ratio, as it can affect bone metabolism. Fe deficiency is still considered to be the most common nutritional deficiency in the world (Haschke and Nosheen, 1991), whereas Zn and Mg deficiency is reflected in mild to severe nutritional symptoms (Hambidge *et al.*, 1986).

During the first month of life, an infant is commonly fed breast milk or a formula. However, by 4–6 months of age, child nutritional requirements increase, so it is necessary to begin supplementary feeding. Weaning foods are the main source of supplementary feeding. They include a wide variety of foods, such as cereals, fruits, vegetables, meat, fish, etc. Industrially produced weaning foods, especially those sold in jars (homogenized weaning foods), are available in a wide range of varieties, which should mean that there is an increase in research into their nutritional quality (Abellán *et al.*, 1994).

The ingredients used in the elaboration of baby foods must be analysed for their mineral content (Rincón *et al.*, 1996). Fish muscle is the main component of many homogenized weaning foods, because it is an important source of protein and minerals. Several authors (Navarro, 1991) considered that the mineral content in fish muscle depends mainly on the species, sex, biological cycle and on the part of the fish which is analysed. Mineral content also depends on ecological factors, such as season, place of development, available amounts of nutrients, temperature and contents of salts in the water. If fish muscle is considered a good source of Ca and P, fish bone is an even richer source of both minerals, and it is technically possible to process the flesh of some fish, such as sole and hake, with the bone, by careful prior homogenization, and thus increase the mineral contents. However, it is necessary to know as exactly as possible the amount of mineral that is available for absorption and utilization, that is to say its bioavailability.

The aim of the present study was to ascertain the influence of incorporating fish bone on the content and the *in vitro* availability of Fe, Zn, Mg, Ca and P in different homogenized fish-based weaning foods.

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MATERIALS AND METHODS

Fish species

The fish species selected for this study, sole (*Solea vulgaris vulgaris*) and hake (*Merluccius merlucius*) were provided by Hero España, S.A. The fish were cleaned and washed with distilled deionized water, and the head, tail and skin were removed before preparing the five samples of minced fish, using muscle as well as bone. The different samples were: sole muscle; hake muscle; a mixture of sole muscle with bone and hake muscle without bone (1:1, w:w); sole muscle with bone; and hake muscle with bone. Each one was used to elaborate a fish purée which would be used later to prepare an experimental weaning food.

Preparation of samples

Figure 1 shows the flow diagram of the different steps followed in the manufacturing process of infant weaning foods. First, the fish muscle samples were minced in a steel hand-mincer (particle size 0.5 cm), and then used to elaborate the fish purées (sole purée without bone (S); hake purée without bone (H); sole with bone and hake without bone purée (SBH); sole purée with bone (SB); and hake purée with bone (HB)) manufactured in a 75% (w/w) dilution in distilled water. The purées were heated in hermetically closed glass bottles in a water bath at 100°C for 5 min. After cooling to room temperature, the fish purées were homogenized and included in the weaning food formulations on a 24% wet weight basis following the ESPGAN recommendations (ESPGAN, 1981). The weaning foods studied were: sole without bone (S); hake without bone (H); sole with bone and hake without bone (SBH); sole with bone (SB); and hake with bone (HB). The other ingredients used to elaborate the weaning foods were added in the proportions shown in Table 1. Weaning foods (250 g bottles) were prepared experimentally by Hero España, S.A. (Alcantarilla, Spain).

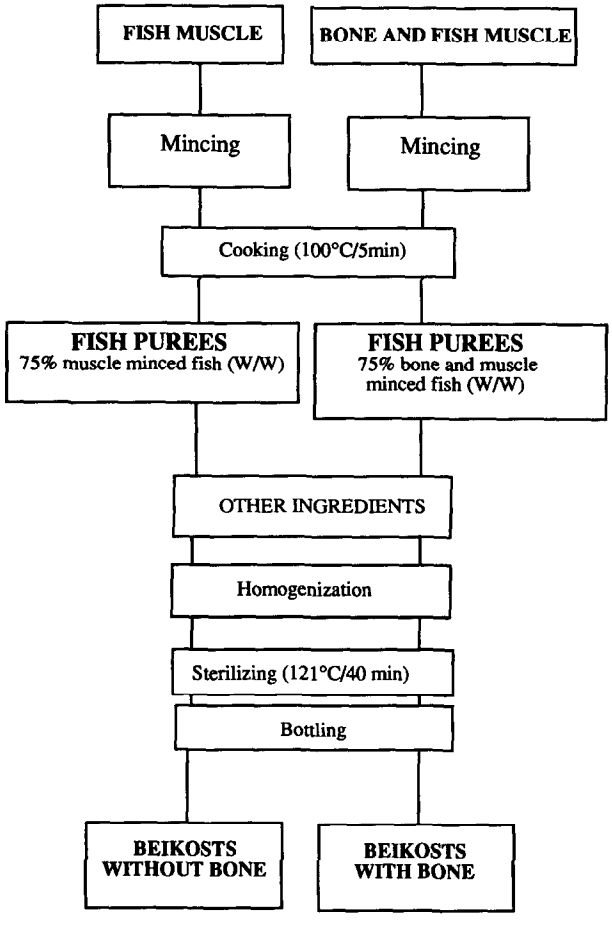

Fig. 1. Flow diagram of weaning food manufacture.

Mineral composition

The Fe, Zn, Mg and Ca contents of the fish purées and weaning foods were analysed after destroying the organic matter of the samples by dry ashing in a Nabertherm furnace oven, model L3/P (Lilienthal, Bremen, Germany) for 12 h with a final temperature of 525°. The ash was dissolved with 2 ml of concentrated HNO_3 acid on a hot plate, and the volume was made up to 50 ml with distilled deionized water. The minerals were measured by flame atomic absorption spectrophotometry, using a Perkin–Elmer AA spectrophotometer model 3100 (Norwalk, CT, USA) with an air–acetylene flame, flow spoiler and corrosion resistant nebulizer. A monoelemental hollow cathode lamp was used for each element. The instrumental conditions recommended by the manufacturer (wavelength, slit and lamp intensity) were applied (Anonymous, 1978). P content was determined after dry ashing, in the same conditions applied for the other minerals, using the ammonium vanadate colorimetric method (Chapman and Pratt, 1961), reading the absorbance in a Hitachi H-2000 double beam molecular spectrophotometer.

Table 1. Ingredients and percentages used for weaning food manufacture

Ingredients	Percentage in Weaning foods
Fish purée[a]	24.00
Potato *(Solanum tuberosum)*	8.15
Butter	2.86
Powdered milk	1.00
Tomato concentrate	1.00
Onion *(Allium cepa)*	0.53
Celery *(Apium graveolens)*	0.53
Salt	0.25
Water (distilled)	61.68

[a]Fish pureé was different for each type of weaning food: sole purée without bone (S); hake purée without bone (H); sole with bone and hake without bone pureé (SBH); sole purée with bone (SB); hake purée with bone (HB).

Mineral *in vitro* bioavailability determination

The proportion of available mineral in the fish purées and weaning foods was determined following the method of Miller *et al.* (1981). This method involves simulated gastrointestinal digestion (pepsin–HCl and pancreatin digestions), followed by the measurement of soluble minerals. One hundred and fifty grams of homogenized samples were adjusted to pH 2.0 with 6 M HCl. To perform the pepsin–HCl digestion, 0.5 g of pepsin (P-700 from hog stomach mucosa, Sigma Chemical Co., St Louis, MO) were added per 100 g of sample. These were incubated in a shaking water bath at 37°C for 2 h. After pepsin digestion, triplicate 40 g samples were transferred to 200 ml plastic bottles. Tightly closed dialysis tubing containing 50 ml of deionized water and an amount of $NaHCO_3$ equivalent to the measured titratable acidity measured previously, were placed in the 200 ml bottles with the samples. The bottles were placed again in a shaking water bath at 37°C for 30 min. To carry out pancreatic digestion, 5 ml of pancreatin–bile extract mixture, 4 g pancreatin (P-1750, from porcine pancreas, Sigma Chemical Co.) and 25 g porcine bile extract (B-87556, Sigma Chemical Co.) per litre of 0.1 M $NaHCO_3$, were added to each bottle and then incubated in a water bath for 3 h at 37°C. Later, the dialysis tubes were removed from the bottles, washed and weighed. The Fe, Zn, Mg and Ca contents of the dialysis tubes were analysed by flame atomic absorption spectrophotometry. The P content was determined using the colorimetric technique (Chapman and Pratt, 1961), after dry-ashing an aliquot of the contents of the dialysis tubes. Mineral *in vitro* availability was calculated as the percentage dialysed of the total amount of the element present in the aliquot (% dialysability). The amount of the minerals dialysed was also estimated per 100 g of sample, calculated from the values of the percentage dialysability and concentration of each element in the sample; (% dialysability $\times 10^{-2}$) $\times$ (mg $100\,g^{-1}$). For each trial, six samples were assayed.

Assay quality control

To minimize the risk of contamination, all glassware and crucibles were washed overnight in a 6 N HNO_3 acid solution. Distilled deionized water was used to rinse all glassware. Community Bureau of reference material CRM-422 (cod muscle) was used as a control to test the method for accuracy and precision, and was analysed together with the samples.

Statistical analysis

Statistical analysis was performed using SYSTAT software, version 5.0 (Wilkinson and Howe, 1992). An analysis of variance (ANOVA) was applied to ascertain the effect of adding bone on the mineral content and *in vitro* availability of fish purées and weaning foods. Tukey's test, with a significance level of 95%, was used to compare individual pairs of means.

RESULTS AND DISCUSSION

Results of the variance analysis made on the contents and dialysed percentages of Fe, Zn, Mg, Ca and P, as affected by bone addition in fish purées and weaning foods, are shown in Table 2. Only Ca and P depended significantly ($p < 0.001$) on the addition of bone in fish purées and weaning foods. The presence of bone affected the dialysed percentage of Fe ($p < 0.01$) and Zn ($p < 0.001$) in fish purées and Zn ($p < 0.05$), Mg ($p < 0.01$) and Ca ($p < 0.001$) in the weaning foods, no significant effect being observed on the percentage of P dialysed.

Mineral content and *in vitro* availability in purées

The mineral contents of purées, expressed as mg $100\,g^{-1}$ on a wet weight basis, are shown in Table 3. The Fe contents were reduced in all the purées assayed, ranging from 0.18 to 0.72 mg $100\,g^{-1}$ for SB and SBH purées, respectively, and not being affected by the incorporation of bone (Table 2). Generally, the percentage of *in vivo* availability of Fe in foods has been considered to be lower than 25% (Fairweather-Tait, 1992). In the current study, available Fe percentages were lower than 5%, decreasing after bone incorporation. So, the values ranged from 0.68 to 4.30% in SBH and S purées, respectively. This may be due to the differences between *in vivo* and *in vitro* methods, because fish protein stimulates the production of gastric acid and thereby promotes the

Table 2. Variance analysis (*F* values and probability) of content and dialysed percentage of Fe, Zn, Mg, Ca and P, for the addition of bone in fish purées and in beikots

	Fe	Zn	Mg	Ca	P
Mineral content (mg $100\,g^{-1}$)					
Fish purées	1.32NS	6.52NS	1.04NS	23.7***	59.8***
Weaning foods	4.52NS	0.47NS	1.29NS	115***	9.48**
Dialysed percentage					
Fish purées	12.9**	81.1***	0.27NS	0.93NS	0.02NS
Weaning foods	1.54NS	8.27*	9.67**	24.7***	1.54NS

Significant differences for: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. NS = no significant differences for $p > 0.05$.

Table 3. Fe, Zn, Mg, Ca and P contents (mean ± standard deviation), expressed as mg 100 g^{-1} on a weight basis, percentage and dialysed amount for fish pureé samples

Minerals	Type of fish purée				
	Without bone			With bone	
	S	H	SBH	SB	HB
Fe					
mg 100 g^{-1}	0.37 ± 0.01^{c}	0.43 ± 0.28^{bc}	0.72 ± 0.13^{a}	0.18 ± 0.05^{d}	0.42 ± 0.09^{bc}
% of DM	4.30 ± 0.35^{a}	2.01 ± 0.54^{b}	0.68 ± 0.19^{c}	1.93 ± 0.75^{b}	1.26 ± 0.45^{bc}
Amount of DM (mg 100 g^{-1})	t	t	t	t	t
Zn					
mg 100 g^{-1}	0.36 ± 0.00^{a}	0.34 ± 0.00^{a}	0.44 ± 0.02^{a}	0.48 ± 0.12^{a}	0.38 ± 0.03^{a}
% of DM	4.96 ± 0.54^{b}	8.22 ± 0.39^{a}	1.52 ± 0.11^{c}	0.47 ± 0.10^{d}	0.49 ± 0.01^{d}
Amount of DM (mg 100 g^{-1})	t	t	t	t	t
Mg					
mg 100 g^{-1}	21.8 ± 2.18^{d}	38.5 ± 1.19^{ab}	32.9 ± 1.11^{c}	32.5 ± 1.31^{c}	34.7 ± 2.32^{bc}
% of DM	22.6 ± 3.97^{ab}	13.5 ± 0.61^{d}	16.6 ± 0.15^{c}	18.1 ± 0.45^{bc}	16.4 ± 1.80^{c}
Amount of DM (mg 100 g^{-1})	4.91 ± 0.62^{a}	5.19 ± 0.27^{a}	5.46 ± 0.14^{a}	5.88 ± 0.21^{a}	5.67 ± 0.77^{a}
Ca					
mg 100 g^{-1}	67.7 ± 9.86^{d}	97.1 ± 10.28^{cd}	232 ± 18.50^{bc}	330 ± 47.99^{b}	512 ± 102.90^{a}
% of DM	8.32 ± 2.21^{a}	2.24 ± 0.67^{c}	5.08 ± 0.56^{b}	4.54 ± 0.21^{b}	2.47 ± 0.28^{c}
Amount of DM (mg 100 g^{-1})	5.63 ± 1.04^{b}	2.18 ± 0.51^{b}	11.7 ± 2.25^{a}	15.0 ± 1.59^{a}	32.6 ± 0.63^{b}
P					
mg 100 g^{-1}	401 ± 5.91^{d}	388 ± 12.16^{d}	629 ± 18.14^{c}	674 ± 0.66^{b}	847 ± 11.58^{a}
% of DM	3.31 ± 0.64^{bc}	5.58 ± 0.15^{a}	2.94 ± 0.07^{c}	6.21 ± 0.07^{a}	3.84 ± 0.10^{b}
Amount of DM (mg 100 g^{-1})	13.3 ± 2.39^{d}	21.6 ± 0.13^{c}	18.5 ± 0.71^{c}	41.8 ± 0.49^{a}	32.6 ± 0.63^{b}

S = sole pureé, H = hake pureé, SBH = sole with bone and hake without bone pureé, SB = sole with bone pureé, HB = hake with bone pureé, DM = dialysed mineral, t = trace (t < 0.01).
[a–e] Different characters in the same row are significantly different ($p < 0.05$).

solubilization of Fe from the fish matrix (Santaella *et al.*, 1997). In addition, approximately 40% of the Fe in fish, as well as in meat and poultry, is heme iron (NRC, 1991), which is much better absorbed (15–35%) than non-heme iron (2–20%), through specific binding sites in the intestinal tract. Moreover, the addition of bone increased the Ca content (Table 3), and it is known that Ca forms insoluble complexes with Fe that decrease Fe availability (Hallberg and Rossander-Hulten, 1993). Dialysed amounts of Fe were considered trace ($t < 0.01$), because of their low content and their low *in vitro* availability. Zn contents ranged from 0.34 mg 100 g^{-1} for H purée to 0.48 mg 100 g^{-1} for SB purée, not being affected by the addition of bone. The percentage of dialysed Zn ranged from 0.47 to 8.22% for SB and H purées, respectively. In this case, the incorporation of bone had a negative effect, since fish purées without bone gave higher values of Zn availability. In all the samples investigated, the percentages of dialysed Zn were lower than the values reported by Fairweather-Tait (1992), who found that Zn availability in foods ranged between 25 and 75%. The dialysed amounts of Zn were considered trace, as mentioned for Fe.

Mg levels ranged from 21.8 to 38.5 mg 100 g^{-1} for S and H purées, respectively, which were the two purées without bone, while the purées with bone showed intermediate values. The Mg content increased significantly after bone incorporation for sole-based purées (S, SBH and SB), whereas no significant differences were observed between the two hake purées (H and HB). The percentage of dialysed Mg was highest in the purée with the lowest Mg content (S purée) and lowest in the purée with the highest Mg level (H purée). However, dialysed amounts of Mg were similar in all the samples, at around 5–6 mg 100 g^{-1}, with no significant differences observed between them.

The Ca content increased significantly after bone incorporation, ranging in the purées with bone from 232 to 512 mg 100 g^{-1} for SBH and HB purées, respectively. As already mentioned, among the three purées containing bone, SBH purée showed the lowest value, probably because this purée only included sole bone, but not hake bone. Moreover, the Ca levels found in HB purée varied greatly (Table 3), because hake bone is more consistent than sole. For this reason, it was more difficult to obtain a good degree of homogeneity in the hake samples, which might have included bone fragments of different sizes, even after passing through the hand mincer. The percentage of dialysed Ca was low in all the samples, ranging from 2.24% for H purée and 8.32% for S purée, and was not influenced by bone incorporation (Table 2). However, the amounts of dialysed Ca were significantly higher in the purées with bone and ranged from 11.7 mg 100 g^{-1} for SBH purée to 32 mg 100 g^{-1} for HB purée, due to the high Ca concentrations.

The P content, percentage and dialysed amount of P in fish purées behaved the same as Ca. High P content was found in the fish purées with bone, ranging from 629 mg 100 g^{-1} for SBH purée to 847 mg 100 g^{-1} for HB purée. The low percentages of P availability obtained in all the fish-based purées, ranged from 2.94 to 6.91 for SBH and SB purées, respectively, but no reference values can be found in the scientific literature and no rigorous recommendations have been established (NRC, 1991).

Mineral content and *in vitro* availability in weaning foods

The mineral contents of the weaning foods, expressed as mg 100 g^{-1} on a wet weight basis, are shown in Table 4. The Fe contents were low in all the weaning foods with values lower than 0.44 mg 100 g^{-1}, because fish, the main ingredient, is a poor resource of this element and no other ingredient was capable of providing any substantial amount of this trace element (Table 1). These results were similar to the data reported by Rincón *et al.* (1990) in the fish-based weaning food denominated 'hake with rice'. The percentage of dialysed Fe was low and close to 10% in all the samples studied, perhaps due to the formation of insoluble complexes with Ca that decrease Fe availability (Fairweather-Tait, 1992), as reported for fish purées. Because of the low Fe content and the low percentage of Fe dialysed, the amounts of dialysed Fe were low in all the weaning foods (lower than 0.05 mg 100 g^{-1}).

Zn levels in weaning foods ranged from 0.22 mg 100 g^{-1} (SBH) to 0.29 mg 100 g^{-1} (HB), while the percentage of dialysed Zn ranged from 18.1% (HB) to 25.7% (H), decreasing after the addition of bone, although it was still higher than the percentage found in the fish-based purées. In general, Zn is absorbed more efficiently than Fe (Fairweather-Tait, 1992). The Zn bioavailability percentage in a whole diet ranges from 1 to 90% (Solomons, 1982), although no data have been found in the scientific literature about Zn availability in fish. Zn absorption varies (Solomons, 1982), owing to dietary factors such as phytic acid and the dietary fibre content of foods (Hallberg, 1994). For this reason, a lower absorption of Zn could be expected in weaning foods than in fish purées, since 10.21% of the weaning foods (Table 1) are vegetables, which include phytic acid in their composition (81 mg 100 g^{-1} for potato and 6 mg 100 g^{-1} for tomato on a wet weight basis) (Harland and Oberleas, 1987). This acid, together with the high Ca levels found in bone-supplemented weaning foods, may have contributed to the formation of Zn–Ca–phytate complexes, thus decreasing Zn availability. However, this is not observed in our experience.

Mg levels found in all the weaning foods investigated were higher than the levels reported by Rincón *et al.* (1990) in the fish-based weaning foods denominated 'hake with rice'. Mg levels ranged from 15.7 to

Table 4. Fe, Zn, Mg, Ca and P contents (mean ± standard deviation), expressed as mg 100 g^{-1} on a weight basis, percentage and dialysed amount for fish-based weaning food samples

Minerals	Type of weaning food				
	Without bone			With bone	
	S	H	SBH	SB	HB
Fe					
mg 100 g^{-1}	0.34 ± 0.01^{ac}	0.44 ± 0.01^{a}	0.26 ± 0.01^{cd}	0.39 ± 0.08^{ab}	0.31 ± 0.01^{bc}
% of DM	10.1 ± 0.07^{b}	9.31 ± 0.17^{b}	9.85 ± 0.98^{b}	9.45 ± 0.02^{b}	11.6 ± 0.53^{a}
Amount of DM (mg 100 g^{-1})	0.03 ± 0.00^{c}	0.04 ± 0.00^{ab}	0.02 ± 0.00^{d}	0.03 ± 0.00^{bc}	0.03 ± 0.00^{c}
Zn					
mg 100 g^{-1}	0.28 ± 0.01^{a}	0.26 ± 0.02^{ab}	0.22 ± 0.01^{bc}	0.26 ± 0.00^{a}	0.29 ± 0.01^{a}
% of DM	19.2 ± 0.16^{bc}	25.7 ± 0.91^{a}	22.0 ± 0.59^{b}	18.6 ± 0.19^{bc}	18.1 ± 0.89^{c}
Amount of DM (mg 100 g^{-1})	0.05 ± 0.00^{a}	0.06 ± 0.00^{a}	0.04 ± 0.00^{a}	0.04 ± 0.00^{a}	0.05 ± 0.00^{a}
Mg					
mg 100 g^{-1}	15.7 ± 0.57^{bc}	17.9 ± 0.93^{a}	15.8 ± 0.02^{bc}	16.2 ± 0.49^{ab}	16.5 ± 0.54^{ab}
% of DM	33.6 ± 0.25^{a}	29.4 ± 0.06^{b}	24.3 ± 1.41^{c}	20.0 ± 0.30^{d}	30.6 ± 1.10^{b}
Amount of DM (mg 100 g^{-1})	5.26 ± 0.15^{a}	5.25 ± 0.27^{a}	3.83 ± 0.29^{b}	3.24 ± 0.29^{b}	5.05 ± 0.30^{a}
Ca					
mg 100 g^{-1}	39.0 ± 4.35^{d}	51.7 ± 1.61^{c}	78.9 ± 1.18^{b}	82.8 ± 4.25^{b}	92.8 ± 0.44^{a}
% of DM	16.3 ± 1.36^{c}	16.1 ± 0.35^{c}	21.4 ± 1.95^{b}	20.3 ± 1.92^{b}	26.0 ± 1.03^{a}
Amount of DM(mg 100 g^{-1})	6.36 ± 0.34^{c}	8.31 ± 0.36^{c}	16.9 ± 1.54^{b}	16.8 ± 2.92^{b}	24.1 ± 0.84^{a}
P					
mg 100 g^{-1}	203 ± 9.22^{c}	195 ± 3.99^{c}	223 ± 10.72^{c}	244 ± 5.44^{ab}	274 ± 1.93^{a}
% of DM	2.15 ± 0.06^{b}	2.72 ± 0.24^{a}	2.41 ± 0.04^{ab}	2.19 ± 0.06^{b}	2.28 ± 0.19^{b}
Amount of DM(mg 100 g^{-1})	4.81 ± 0.54^{bc}	5.31 ± 0.52^{ab}	5.40 ± 0.21^{ab}	5.36 ± 0.07^{ab}	6.10 ± 0.52^{a}

S = sole weaning food, H = hake weaning food, SBH = sole with bone and hake without bone weaning food, SB = sole with bone weaning food, HB = hake with bone weaning food, DM = dialysed mineral.
$^{a-e}$ Different characters in the same row are significantly different ($p < 0.05$).

17.9 mg 100 g^{-1} in the two weaning foods without bone (S and H weaning foods), while the weaning foods with bone showed intermediate values in the range. The dialysed percentages of Mg ranged from 20.0 to 33.6% for SB and S weaning foods, respectively. Therefore, bone incorporation decreased the dialysed percentage of Mg in sole-based weaning foods (SBH and SB), but not in hake-based weaning foods, as observed in the ANOVA (Table 2).

As expected, Ca levels increased after bone incorporation, as a result of the Ca content of the mineral matrix of fish bone, ranging from 78.9 mg 100 g^{-1} in SBH to 92.8 mg 100 g^{-1} in HB weaning foods. The percentage of dialysed Ca was higher in weaning foods than in purées (Table 3), probably because the powdered milk used in weaning food formulation (Table 1) is rich in lactose, which is one of the factors that promote Ca availability (Gueguen, 1990). Also, the percentage and amount of dialysed Ca were higher in the weaning foods with bone than in those without it, due to their higher Ca content. The hake-based weaning food with bone (HB) showed higher values in all the parameters assayed for Ca than the sole-based weaning food with bone (SB), perhaps the larger size and greater consistency of hake bone result in a greater contribution from the bone matrix, as mentioned for fish purées.

Bone incorporation affected P levels ($p < 0.05$), but not the percentage of P dialysed (Table 2). P levels were significantly higher in all the samples with bone, although there were no significant differences between them. The dialysed percentage of P was low in all the samples and ranged from 2.15 to 2.72 for S and H weaning foods, respectively, but there were no significant differences between the weaning foods with or without bone added. The amounts of dialysed P were slightly higher in the samples with bone, but without significant differences between any samples assayed.

From a nutritional point of view, the same amount of Ca and P (1/1) should be ingested; although a molar Ca/P ratio of 1/1.5 is acceptable, injurious effects appear when this relation is inverted (1/2) (Aranda and Llopis, 1993). From the Ca and P levels found in the samples, the Ca/P ratios were calculated, as it is important to maintain a good ratio for optimal bone mineralization. The Ca/P ratio increased after bone addition and very similar ranges (0.30–0.37) were obtained for all the weaning foods with bone.

Figure 2 represents the estimation of the amounts of dialysed Mg, Ca and P from fish purées and from the other ingredients that composed the weaning foods. The amounts of dialysed Fe and Zn were not represented, as these values provided by fish purées were insignificant, as shown in Table 4. The dialysed amounts of Mg from the fish pureés were lower than the amounts provided by the other ingredients, the only exception being the hake with bone weaning food (HB). Furthermore, the dialysed amount of Mg from fish purées decreased after bone incorporation, which may, therefore, be regarded

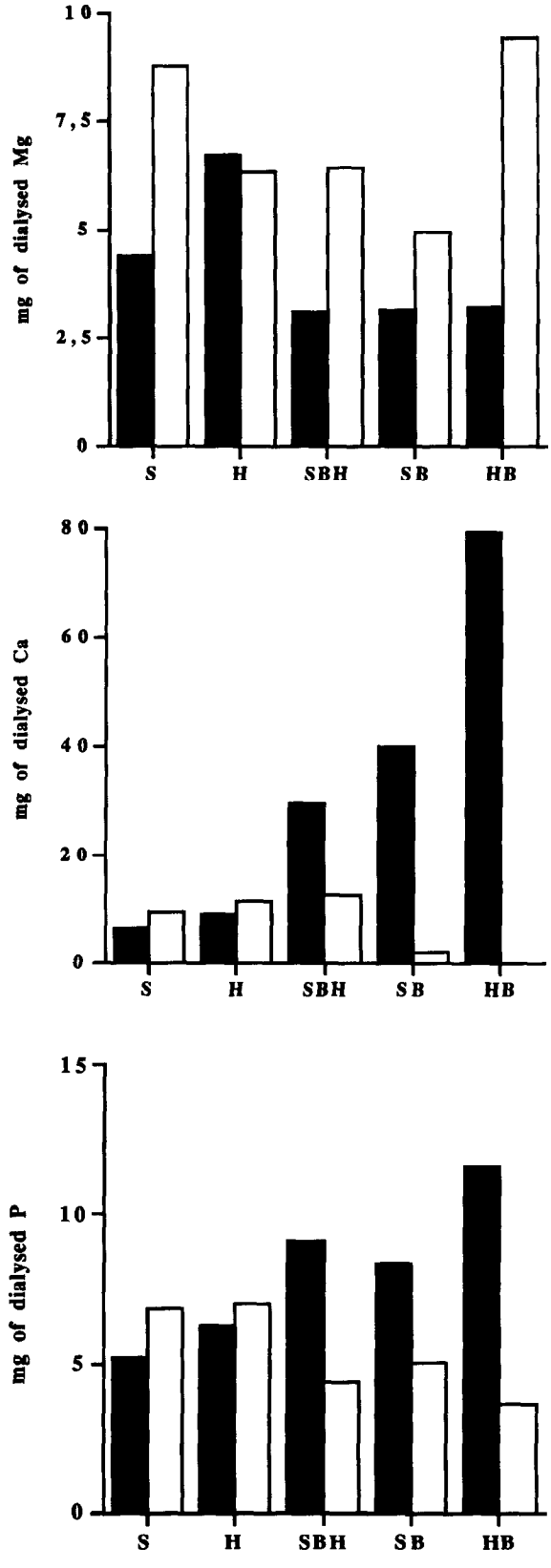

Fig. 2. Graphic representation of amount of Mg, Ca and P dialysed from the fish purée (■) and from the rest of the ingredients (□) used to elaborate the infant weaning foods: sole without bone (S); hake without bone (H); sole with bone and hake without bone (SBH); sole with bone (SB); hake with bone (HB).

as decreasing Mg *in vitro* availability. The addition of bone led to increased amounts of Ca being dialysed from the purées, these amounts being higher than those dialysed due to the other ingredients. For the hake-based weaning food with bone (HB), we observed that all the dialysed Ca was due to the Ca provided by the purées. The amounts of P dialysed in weaning foods with bone were due to the purées used rather than the other ingredients. From this, we inferred that the incorporation of bone increased, not only Ca and P content, but also the *in vitro* availability of these minerals.

CONCLUSION

The five fish weaning foods investigated could not be considered to be good sources of Fe and Zn. Mg levels in all the weaning foods were practically invariable, presenting acceptable levels in all the samples. Homogenized weaning foods containing both fish muscle and bone presented higher Ca and P levels and higher available amounts of these minerals, due principally to the fish purées used in their formulation. To summarize, the incorporation of bone improved the Ca and P contents, although the levels of Fe and Zn remained low in all the samples assayed.

ACKNOWLEDGEMENTS

The authors acknowledge the grant project CYCIT ALI 94-0338 of the Spanish Government, and a research grant from Hero, Spain S.A.

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