

Effect of wheat bran and pectin on the absorption and retention of phosphorus, calcium, magnesium and zinc by the growing pig.

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Summary. Two separate balance experiments of P, Ca, Mg and Zn were carried out on 5 lots of 4 growing pigs each (35-40 kg) adapted for 3 weeks to one of the diets studied. In the first experiment, the control diet was compared with a diet containing 20 % of coarse wheat bran, thus richer in minerals, the quantities ingested not being equalized. In the second experiment, three diets were compared : a control diet, a diet with 2.5 % of high-methoxylated (HM) apple pectin, and a diet with 2.5 % low-methoxylated (LM) apple pectin.

The supplement of P and Mg provided by the wheat bran was well absorbed (apparent absorption) and retained by the pigs. On the contrary, in spite of higher intake of Ca and Zn with bran diet, the absorption of these minerals was not improved. The action of wheat bran phytase and the possible absorption of P and Mg (but not of Ca and Zn) in the large intestine could explain these results.

Compared to HM pectin that had relatively little effect on mineral utilization, LM pectin drastically diminished the absorption and retention of the minerals studied and resulted in negative Ca, Mg and Zn balances. The degree of pectin esterification would thus be the main factor determining the effect of pectin on mineral availability.

In conclusion, wheat bran is a source of available P and Mg for the pig but it might have an unfavorable effect on the utilization of Ca and Zn. LM pectin produces a deleterious influence on mineral balances.

Introduction.

The well-known beneficial action of dietary fibers should not mask their putative unfavorable effects on the biological availability of some nutrients, especially minerals. Since the earliest work of Mellanby on dogs in 1921, many authors have shown the rachitogenic effect in humans and domestic animals of diets with high levels of whole cereals. The factor responsible was soon identified as phytic acid, abundant in seed envelopes ; this was confirmed in many balance experiments beginning with McCance and Widdowson in 1942 and continuing with Walker, Fox and Irving in 1948. More than 20 years later, Reinhold (1971) showed multiple mineral deficiencies, particularly of zinc, in rural populations in southern Iran which ate whole, unleavened bread, and Wills *et al.* (1972) attributed to phytic acid the rickets found among certain immigrants in England.

The inhibitory effect of the properly so-called fibers of cereals on the utilization of minerals has been reported by Phandis and Sohonie (1974) and by Reinhold *et al.* (1975), suggesting that fibers rather than phytates determined the availability of minerals, while Davies, Hristic and Flett (1977) and Andersson *et al.* (1983), again attributed the deleterious effect of cereal diets to phytic acid. Nevertheless, the work *in vitro* of James, Branch and Southgate (1978), showing the strong binding capacity of ionized carboxyl groups of uronic acids, gives an explanation for the effect on minerals of other constituents of dietary fibers, such as hemicelluloses and pectins.

As regards human nutrition, the practical importance of this fiber-mineral interaction has not yet been elucidated, partly due to the small number of balance experiments carried out on humans and also due to the difficulty in extrapolating to humans results obtained in rats. In the latter, the phytase of the intestinal mucosa (Bitar and Reinhold, 1972) or that of bacteria of the large intestine (Wise and Gilbert, 1982) is known to hydrolyze phytates, which would explain why wheat bran does not always have an unfavorable effect in this species (Bagheri and Guéguen, 1981, 1982). Pigs, which have no intestinal phytase (Pointillart, Fontaine and Thomasset, 1984) and show signs of Ca and Zn deficiency when the diet contains a high level of phytates, are thus better models for mineral studies applied to human nutrition.

The first experiment of the present study tried to investigate and explain the effect of wheat bran on the utilization of P, Ca, Mg and Zn in pigs. As bran is also rich in some minerals and as this supply could be beneficial, we compared a diet containing 20 % of bran with a control diet which we purposely did not supplement with minerals. Accordingly, it attempted to estimate the efficiency of the intrinsic minerals of the bran and the effect of this supply on mineral balances.

The effect of pectin on mineral metabolism is not as well-known and, to our knowledge, has never been studied in pigs. A second experiment was thus designed to compare the effects of two pectins of different degrees of esterification at the same mineral intakes.

The results obtained have already been partially and briefly published (Bagheri and Guéguen, 1983) but have never been presented in full.

Material and methods.

1. *Wheat bran and pectins.* — The standard wheat bran was prepared by « Les Grands Moulins de Paris » and the mean size of the particles was 660 μm . Its main constituents (Brillouet and Thibault, 1981) were, in % of raw product : water 9.8 and, in % of dry matter : ash 6.6, protein (N \times 5.7) 13.6, starch 11.3, pentose polymers 22.9, cellulose 10.0, lignin 7.9. It contained, in g per kg dry matter : total P 12.9, phytic P 8.9, Ca 1.0, Mg 4.7 and Zn 0.073.

The pectin (Unipectine, Paris), extracted from apple pulp, had two different degrees of esterification : 72.5 % for the high-methoxylated (HM) pectin and

39.9 % for the low-methoxylated (LM) pectin. The chemical characteristics of the two pectins (Brillouet and Thibault, 1981) are given in table 1.

TABLE 1
Characteristics of the pectins (in %).

	HM pectin	LM pectin
Water	8.9	9.7
Protein (N × 6.25)	1.5	0.7
Neutral sugars	21.7	13.9
Anhydrogalacturonic acids	63.7	64.9
Methoxyls	8.1	4.6
Degree of esterification	72.5	39.9

2. *Diets.* — The formulas and analytical mineral compositions of the diets used are given in tables 2 and 3, respectively. The control diet was a conventional mixture of corn and soybean meal, to which 20 % of wheat bran was added (experimental diet) at the expense of the corn. To facilitate the interpretation of the results on bran mineral utilization, the corresponding control diet was not supplemented with minerals, except for sodium and calcium which were too low ; the latter however was not added sufficiently, to avoid an increase in Ca:P imbalance. Therefore, Ca and P levels in the control diet (table 3) were lower than the French recommendations for growing pigs (Guéguen and Perez, 1981) but were sufficient for a limited period of normal growth. The bran diet thus had a much higher mineral content than the control diet.

TABLE 2
Diet formulas (%).

	Exp. I		Exp. II		
	Control I	Coarse bran	Control II	HM pectin	LM pectin
Corn	77.5	57.0	75.5	74.0	74.0
Soybean	20.0	20.0	20.0	20.0	20.0
Coarse bran		20.0			
Pectin (HM)				2.5	
Pectin (LM)					2.5
Mineral mixture I	1.5	2.0			
Mineral mixture II			3.5	3.5	3.5
Vitamin mixture	1.0	1.0	1.0	1.0	1.0

Mineral mixture I : CaCO_3 : 80 % ; NaCl : 20 %.

Mineral mixture II : CaCO_3 : 40 % ; $\text{CaHPO}_4 \cdot 2 \text{H}_2\text{O}$: 43 % ; NaCl : 15 % ; $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$: 1.0 % ; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$: 0.8 % ; $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$: 0.2 %.

Vitamin mixture supplying for 100 kg of diet : Vitamin A : 500 000 IU ; vitamine D_3 : 100 000 IU ; Vitamin E : 1 500 IU ; thiamin hydrochloride : 0.2 g ; riboflavin : 0.5 g ; ascorbic acid : 2.0 g ; vitamin B_{12} : 2.5 g ; niacin : 2.0 g ; calcium pantothenate : 2.0 g ; choline : 50 g.

Apple pectin was added into the diet at a rate of 2.5 %, a preliminary experiment having shown that higher amounts caused diarrhoea. Since pectin, unlike bran, does not contain minerals, all the diets were completed with an

equilibrated mineral mixture. Hence, the mineral levels in the three diets (table 3) conformed to dietary recommendations (Guéguen and Perez, 1981).

TABLE 3
Mineral composition of the diets.

	Exp. I		Exp. II		
	Control I	Bran (20 %)	Control II	HM pectin	LM pectin
P, g/kg	3.82	5.58	6.58	6.41	6.88
Ca, g/kg	5.90	8.18	10.17	8.54	8.50
Mg, g/kg	1.48	2.38	1.40	1.14	1.09
Zn, mg/kg	40	61	138	118	126

3. *Animals and experimental protocol.* — For the two separate experiments, twenty male Large White pigs with an average initial weight of 35 kg were used : 2 groups of 4 each for the bran experiment and 3 groups of 4 each for the pectin experiment. The animals were adapted during 3 weeks to one of the preceding diets, then put into individual steel metabolism cages ; the urine and feces were collected separately.

The diets were distributed in two meals per day in limited quantities to avoid refusals. During the balance period, the amounts of intake were 1 200 to 1 250 g (control diet) and 1 000 to 1 200 g (control diet) of dry matter per day in the bran and the pectin experiment, respectively. Distilled water was available *ad libitum*.

The amounts ingested and excreted were measured every day for 10 consecutive days and fecal samples were dried at 70 °C, then ground. Various assays were carried out on the representative samples of the 10-day collections.

4. *Analytical methods.* — P, Ca, Mg and Zn were assayed on samples of diet, feces or urine after ashing in a muffle furnace at 530 °C for about 8 h. The ash was dissolved with pure HCl then, after complete evaporation, was put into a 10 % HNO₃ solution. After filtration, the solutions were diluted according to the concentration of the element to be assayed.

Phosphorus was determined by ammonium phospho-vanadate colorimetry, Ca by flame spectrophotometry (Eppendorf) and Mg and Zn by atomic absorption spectrometry (IL 151). Phytic P was determined by a modification of the method of Oberleas (1971).

5. *Statistical analysis.* — The Newman-Keuls test was used for comparison of the means after variance equality had been checked by the Bartlett χ^2 -test (Snedecor and Cochran, 1967).

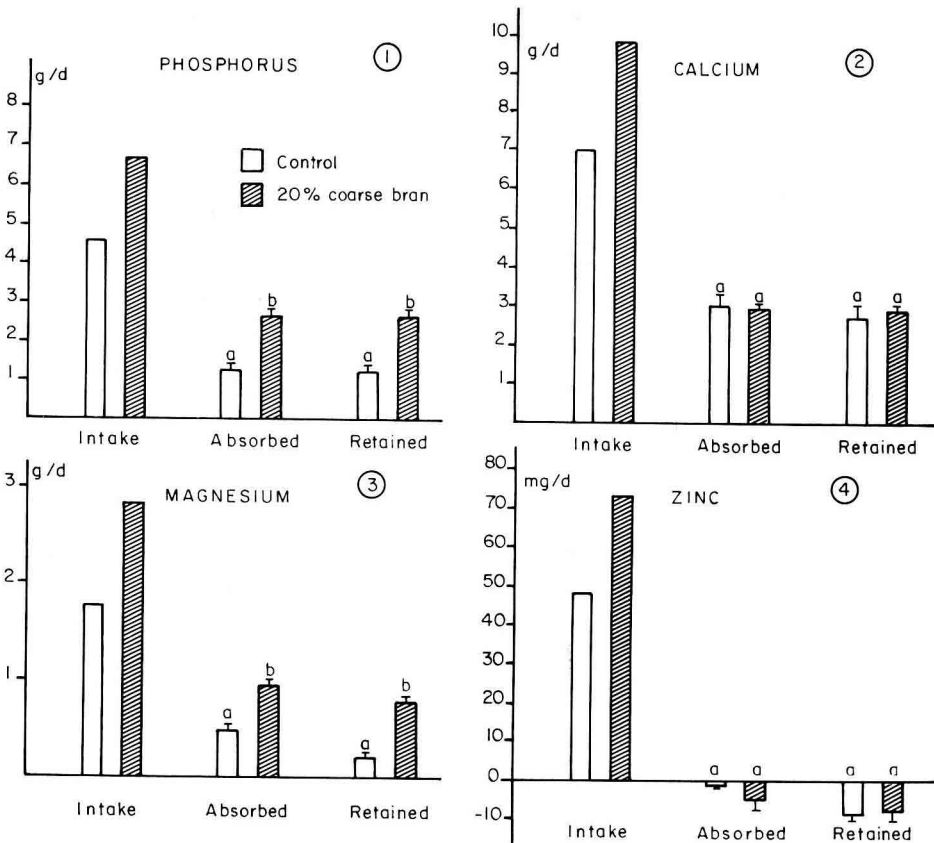
Results.

1. *Effect of wheat bran.* — After an adaptation period of 3 weeks, the behavior and appetite of the pigs were normal and the average weight gain during the balance period was 475 and 712 g/day for the control and 20 % bran groups, respectively.

The amounts of P, Ca, Mg and Zn ingested, absorbed (apparent absorption) and retained are shown in figures 1, 2, 3 and 4, respectively. The corresponding values of the percentages of apparent absorption and retention are given in table 4. Urinary excretion was negligible for P and Ca but more important for Mg and Zn.

The additional supplies of P and Mg provided by bran were well absorbed and retained (figs. 1, 3). Furthermore, in spite of the higher supplies of P and Mg, the percentages of apparent absorption (AA %) and retention (R %) were higher with bran and the urinary excretion of Mg was lower.

On the contrary, the additional Ca supplied by the 20 % of bran did not result in increased absorption and retention of this mineral (fig. 2). AA % and R % (table 5) dropped to abnormal levels in the presence of bran, though ingested values were not excessive. The bran considerably increased Zn ingestion but did not change its absorption and retention, the Zn balance not being significantly modified (fig. 4). The urinary excretion of Zn was lower with the bran diet.



FIGS. 1, 2, 3, 4. — Effect of wheat bran on the utilization of phosphorus, calcium, magnesium and zinc in growing pigs.

$M \pm SEM$; $n = 4$.

Values with the same superscript are not significantly different at $P \leq 0.05$.

TABLE 4

Effect of wheat bran on the percentage of apparent absorption (AA %) and retention (R %) of minerals in pigs.

		P	Ca	Mg	Zn
Intake, g/d	Control	4.58	7.11	1.78	0.048
	+ Bran	6.70	9.85	2.85	0.073
% Absorbed	Control	27.5 ± 3.4 ^a	42.8 ± 4.5 ^a	28.1 ± 3.1 ^a	< 0 ^a
	+ Bran	39.7 ± 2.3 ^b	30.0 ± 1.0 ^b	33.2 ± 1.7 ^a	< 0 ^a
% Retained	Control	26.9 ± 3.4 ^a	38.2 ± 4.0 ^a	12.6 ± 1.9 ^a	< 0 ^a
	+ Bran	39.3 ± 2.3 ^b	29.3 ± 1.1 ^b	27.5 ± 2.2 ^b	< 0 ^a

M ± SEM (n = 4).

Values in the same column with different superscripts are significantly different at $P \leq 0.05$.

2. *Effect of pectin.* — The amounts of the diets ingested spontaneously by the pigs were decreased from 1 200 for the control group to 1 000 g per day for the two pectin groups to avoid refusals. This explains the lesser amounts of minerals ingested in the two experimental groups. Average daily weight gain during the balance period was 675, 475 and 650 g for the control, HM pectin and LM pectin groups, respectively.

The amounts of P, Ca, Mg and Zn ingested, absorbed (apparent absorption) and retained are shown in figures 5, 6, 7 and 8, respectively. The corresponding AA % and R % values are given in table 5. Urinary excretion was always very low for P and Ca but relatively high for Mg and Zn.

Considering the differences in the amounts ingested, it appears that incorporating 2.5 % of HM pectin into the diet did not markedly change the utilization of the studied minerals. The decrease in the amounts of Ca and Mg absorbed and retained expressed the decrease in the amounts ingested (figs. 6, 7). On the other hand, the LM pectin highly significantly diminished the apparent absorption and retention of Ca and Mg ($P \leq 0.01$) and decreased P and Zn absorption ($P \leq 0.05$), resulting in negative Ca, Mg and Zn balances.

TABLE 5

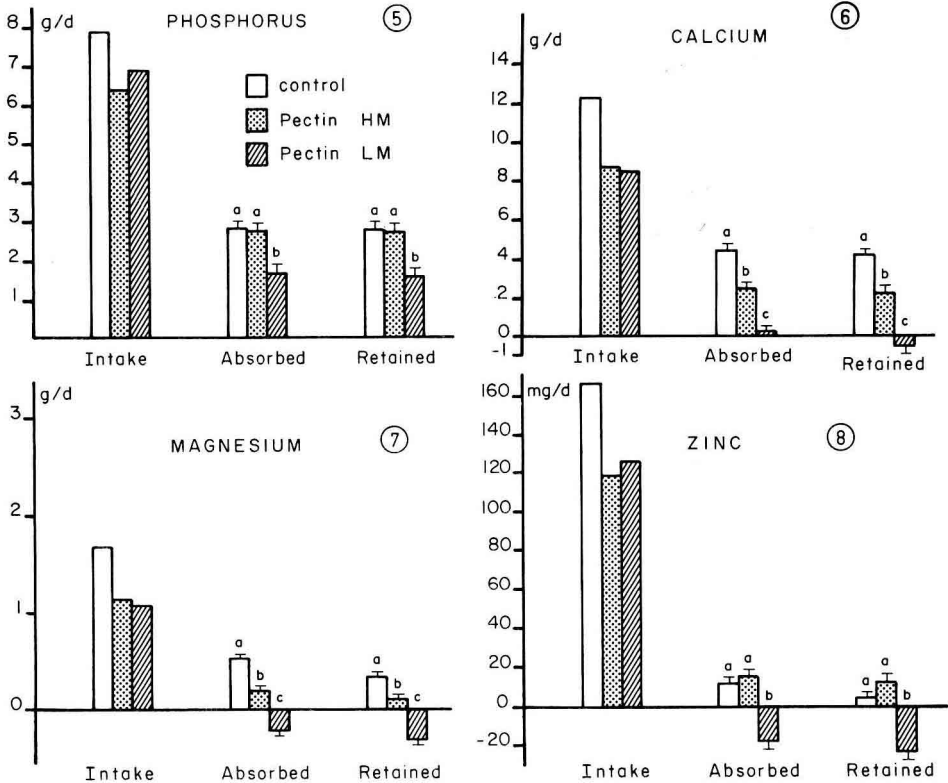
Effect of apple pectin on the percentages of apparent absorption (AA %) and retention (R %) of minerals in pigs.

		P	Ca	Mg	Zn
Intake, g/d	Control	7.90	12.23	1.68	0.165
	+ Pectin HM	6.41	8.70	1.14	0.118
	+ Pectin LM	6.81	8.57	1.08	0.125
% Absorbed	Control	36.0 ± 2.1 ^{ab}	36.8 ± 1.9 ^a	31.7 ± 1.3 ^a	7.4 ± 4.7 ^a
	+ Pectin HM	43.6 ± 2.9 ^a	28.1 ± 3.8 ^a	17.8 ± 2.9 ^a	12.3 ± 5.9 ^a
	+ Pectin LM	24.8 ± 5.7 ^b	0.42 ± 7.5 ^{b*}	< 0 ^{b*}	< 0 ^b
% Retained	Control	35.6 ± 2.1 ^{ab}	34.2 ± 2.0 ^a	20.5 ± 2.4 ^a	2.8 ± 2.7 ^a
	+ Pectin HM	42.0 ± 5.4 ^a	27.4 ± 3.8 ^a	10.1 ± 3.3 ^a	11.0 ± 6.1 ^a
	+ Pectin LM	23.8 ± 5.7 ^b	< 0 ^{b*}	< 0 ^{b*}	< 0 ^b

M ± SEM (n = 4).

Values in the same column with different superscripts are significantly different at $P \leq 0.05$.

Values with different superscripts and an asterisk are significantly different at $P \leq 0.01$.



FIGS. 5, 6, 7, 8. — Effect of apple pectin on the utilization of phosphorus, calcium, magnesium and zinc in growing pigs.
 M ± SEM ; n = 4.
 Values with the same superscript are not significantly different at P ≤ 0.05.

Discussion.

1. *Effects of wheat bran.* — Since the main object of this experiment was the study of the effects on P, Ca, Mg and Zn balances of a high supply (20 %) of wheat bran, rich in minerals, the control diet contained no supplement of inorganic salts to equalize the amounts ingested. It is thus difficult to compare the percentages of absorption and retention (table 4). Nevertheless, the present results confirm the well-known fact that, in pigs, contrary to humans, with a normal intake of Ca and P, urinary excretion of these two minerals is negligible since retention almost equals apparent absorption ; however, this is not true for Mg and Zn.

The values obtained (figs. 1, 2, 3, 4) clearly demonstrate that pigs can utilize the P and Mg of wheat bran and that adding 20 % of bran considerably improved these mineral balances. On the other hand, increasing supplies of Ca and Zn by the

bran diet did not ameliorate their retention. The negative Zn balance obtained with the two diets shows that not only was the Zn level (40 ppm) of the control diet insufficient but the Zn supplement provided by bran was not available.

Many studies on pigs have shown that wheat and wheat bran P are much better utilized than corn P but not as well utilized as inorganic P (Miracle *et al.*, 1977 ; Pierce *et al.*, 1977 ; Stober *et al.*, 1979 ; Cromwell, 1979 ; Pointillart, Fontaine and Thomasset, 1984). The scale of the relative biological efficiency of P, established by Cromwell (1980) in relation to bone breaking resistance in growing pigs, assigns values of 0.48, 0.29 and 0.12 to wheat, wheat bran and corn, respectively, compared to the reference value of 1 attributed to monosodium phosphate. The true absorption percentage of wheat bran P labelled with ^{32}P was estimated at 37 vs 70 for disodium phosphate (Guéguen, Besançon and Rérat, 1968). Wheat bran P is thus much better utilized by pigs than anticipated and this was confirmed by the work of Newton, Hale and Plank (1983) on pigs receiving longer-term (80-day) diets enriched with wheat bran. These authors noted an obvious decrease in P and Mg levels in the undigestible fraction of bran in the feces.

Our results also show that not only is wheat bran P well absorbed, but the presence of bran improves the utilization of P of the corn-soybean diet. This confirms the observation of Collings *et al.* (1979) on pigs receiving a diet enriched with wheat middlings and might be explained by bran phytase (E.C. 3.1.3.8) intake (Pointillart, Fontaine and Thomasset, 1984).

Fewer studies have been carried out on the influence of wheat bran on Ca and Zn utilization in pigs. Our results concerning the unfavorable effect of bran on the percentage of apparent absorption of Ca (table 4) agree with those of Nicolaysen and Njaa (1951) and the more recent ones of Collings *et al.* (1979). If, on a long-term basis (80 days), Newton, Hale and Plank (1983) found no significant effect on apparent Ca absorption when 10 or 20 % of wheat bran was incorporated, they did discover that Ca accumulated in the undigestible part of the bran recovered in the feces of the pigs. These authors also found a decrease in apparent Zn absorption and a high accumulation of Zn in the undigestible fraction of bran recovered in the feces. Moreover, the negative influence of phytic acid, abundant in bran, on Zn absorption is well known (Oberleas *et al.*, 1962) and could be one of the main factors producing parakeratosis in pigs.

While the bran improves P and Mg utilization it decreases that of Ca and Zn. The fact that P and Mg contents of bran are high may, to some extent, account for the amelioration of the balance of these two minerals. Furthermore, *in vitro* experiments show that bran binds Ca in high amounts but can only bind small amounts of P (Bagheri and Guéguen, unpublished data).

The diets containing 20 % of bran increased cecal volume and the production of volatile fatty acids, favoring the release of the previously bound minerals. Indeed, the proportion of ultrafilterable Ca in the cecum increased by 2 to 3-fold with the bran, while the ultrafilterable Mg concentration remained high in the cecum and was not modified by the presence of bran (Guéguen, Bagheri and Rérat, 1981). Consequently, the overall effect of bran on mineral absorption also probably depends on the ability of the large intestine to absorb eventual

available elements in the cecum. The capacity of the large intestine to absorb Mg is always high (Partridge, 1978a, b). For the other elements, we have shown (Guéguen, Bagheri and Rérat, 1981) that the hind gut absorbed a large part (28 %) of a dose of soluble ^{32}P administered into the cecum, while absorption of ^{45}Ca and ^{65}Zn was low (8.5 and 4.0 %, respectively).

It is tempting to extrapolate to humans the above results on pigs, neither species having intestinal active phytase. Many studies have demonstrated the unfavorable effect in man of the ingestion of wheat bran or whole wheat bread on the utilization of Ca (McCance and Widdowson, 1942 ; Reinhold *et al.*, 1976 ; Campbell *et al.*, 1976 ; Cummings *et al.*, 1979) and Zn (Reinhold, 1971 ; Sandström *et al.*, 1980 ; Sandberg *et al.*, 1982). Zoppi *et al.* (1982) warned the risks of ingesting high doses of bran which have a negative effect on the utilization of some minerals in children. This problem is less serious, at least as concerns Ca, when the diet includes enough dairy products (Sandstead *et al.*, 1979), Ca balance then being little modified by the bran intake. However, as noted by Van Dokkum, Wesstra and Schippers (1982), the absence of effect of bran on mineral balance may simply be a result of increased dietary intake and not mean that the bran has no negative effect on mineral availability. Their results obtained on young adult men and using an experimental design similar to that of the present study, i.e. without mineral supplementation to equalize the intakes, also show that increasing mineral consumption with bran does not ameliorate Ca and Zn balances. Nevertheless, in disagreement with the results obtained in pigs, the very high intake of Mg with the bran-rich diets did not increase the retention of this mineral. This discrepancy might be due to the much higher contribution of bran to the total Mg intake with the human diets.

In conclusion, by increasing available P intake and decreasing Ca absorption, a bran-rich diet may aggravate the traditional Ca:P imbalance of human diets (Guéguen, 1982) and it might be recommended to use dephytinized bran products (Andersson *et al.*, 1983), or to add calcium salts to such a diet.

2. Effect of pectin. — Incorporating 2.5 % of HM pectin into the diet did not markedly change the utilization of the minerals studied. In spite of a lower intake the amounts of P and Zn absorbed and retained were unchanged (figs. 5, 8). Moreover the decrease in absorbed and retained Ca and Mg paralleled the decrease in the ingested amounts (figs. 6, 7).

Compared to HM pectin, at equal mineral intakes, the LM pectin considerably decreased the absorption and retention of the minerals, giving negative Ca, Mg and Zn balances. Considering that the intakes of Mg and Zn were high and exceeded the requirements, these negative balances are quite abnormal. The zero or negative values of apparent absorption of Ca, Mg and Zn obtained with LM pectin are the expression of a large fecal endogenous loss. It is probable that the re-absorption of the endogenous cations in the intestine is also hindered by the presence of LM pectin.

Since dietary fibers can bind Ca ions in direct proportion to their uronic acid content (James, Branch and Southgate, 1978), pectins might be expected to have a high affinity for Ca. Incubation trials using radioisotopes (Bagheri and

Guéguen, unpublished data) have evidenced that the binding percentage is higher for Ca with LM (94 %) than with HM (59 %) pectin.

The effect of degree of pectin esterification could explain the absence of influence on mineral metabolism noted in the scarce studies done on humans (Cummings *et al.*, 1979 ; Drews *et al.*, 1979 ; Lei *et al.*, 1980 ; Sandberg *et al.*, 1983). According to Cummings *et al.* (1979), the lack of effect would be due to degradation of the pectin in the intestine, releasing previously retained minerals, or to the high esterification of the pectin. The latter explanation is the most plausible because the pectin used in their experiment was about 80 % methoxylated. Furthermore, it is hardly likely that minerals, especially Ca and Zn, would be well absorbed in the large intestine.

It thus seems prudent in human nutrition to avoid using low-esterified pectins which have a very unfavorable influence on mineral metabolism.

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Résumé. *Effet du son de blé et de la pectine sur l'absorption et la rétention du phosphore, du calcium, du magnésium et du zinc par le porc en croissance.*

Dans deux expériences distinctes sur des lots de 4 porcs en croissance, pesant de 35 à 40 kg, des bilans de P, Ca, Mg et Zn ont été réalisés après adaptation durant 3 semaines à chacun des régimes étudiés. Dans la première expérience un régime témoin à base de maïs et de tourteau de soja a été comparé à un régime contenant 20 % de son de blé grossier (aux dépens du maïs), et donc plus riche en minéraux, les quantités de minéraux ingérées n'étant pas égalisées. Dans la seconde expérience, trois régimes ont été comparés : un régime témoin, un régime contenant 2,5 % de pectine de pomme fortement estérifiée (HM) et un régime contenant 2,5 % de pectine de pomme faiblement estérifiée (LM).

Le supplément de P et de Mg fourni par le son de blé est bien absorbé (absorption apparente) et retenu par le Porc. Par contre, malgré un apport alimentaire plus élevé, l'absorption de Ca et Zn n'est pas augmentée dans les lots recevant du son de blé. Ces résultats peuvent être expliqués par l'action de la phytase du son de blé et par la possibilité d'absorption de P et surtout de Mg (mais pas de Ca et Zn) dans le gros intestin.

Comparée à la pectine HM qui agit relativement peu sur l'utilisation des minéraux, la pectine LM diminue très fortement l'absorption et la rétention de tous les éléments étudiés et conduit notamment à un bilan négatif de Ca, Mg et Zn. Le degré d'estérification de la pectine serait donc le principal facteur déterminant son effet sur la disponibilité des minéraux.

En conclusion, le son de blé est une bonne source de P et de Mg pour le Porc, et probablement aussi pour l'Homme, mais il peut avoir un effet défavorable sur l'utilisation de Ca et Zn. La pectine faiblement estérifiée a une influence néfaste sur les bilans minéraux.

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