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The relative biological availability of phosphorus in feed phosphates for broilers (*)

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Summary

The relative biological values (R.B.V.) of the following feed phosphates were investigated: two monocalciumphosphates (MCP), two hydrated dicalcium phosphates (DCP), two anhydrous dicalciumphosphates (AnDCP), two defluorinated rock phosphates (DFP), one Ca-Mg-Na-phosphate (CMP), one mono-Na-phosphate (MSP), one di-Na-phosphate (DSP) (= reference P-source), a meat-and-bone meal (MBM) and a Ca-Al-Fe-phosphate (CAP). Two successive experiments were carried out: an increasing Ca: P-ratio (1e experiment) or a constant Ca-level (2e experiment). The effect of the Na: Cl-ratio upon the R.B.V. also received attention. The features of the tibia (breaking strength, ash-content, ash-percentage and P-content) were only considered to calculate the R.B.V.

The broilers were kept in cages, under normal environmental circumstances (deionized water). The P-deficient experimental rations were equally fed to all the uniform test groups during a period of intense mineral anabolism (7-20 days).

The results of both experiments were very similar to each other (1-8 p. 100). Averaged for the two experiments, the following R.B.V. were found in comparison to DSP(= 100): MCP ≠ A (source A) = 98, MCP ≠ B = 93, DCP ≠ A = 101, DCP ≠ B = 92, An DCP ≠ A = 86, An DCP ≠ B = 86, DFP ≠ A = 96, DFP ≠ B = 95, CMP = 102, MSP = 96, MBM = 90 and CAP = 15 p. 100. Clear differences in ammonium citrate-(2 p. 100) solubility do not correlate with analogous R.B.V.-differences. The P-utilization (retention) was markedly favoured by an increasing Na: Cl-ratio in the feed, combined with a metabolizable anion.

Introduction

The great progress made in recent years in the field of feed formulation is first of all due to a better knowledge of the chemical composition of the feed ingredients and more recently to a better qualitative evaluation of the raw materials, e.g. the feed phosphates. These P-sources have to be add to the rations, because

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the (available) phosphorus-supply of plant materials is insufficient to meet the phosphorus-requirements of broilers.

The methodology to determine the (relative) biological utilization of feed phosphates is very complex. Originally the aim was to find fast and simple techniques, e.g. chemical in vitro-techniques (GILLIS, NORRIS and HEUSER, 1948; ANDERSON, CREUG and BURROUGHS, 1966; NELSON and MILES, 1973). However, it became evident that there was a very slight correlation with in vivo-experiments. Consequently to obtain more realistic information, biological tests had to be relied upon. As a result of the diversity of the methods applied, only the most interesting tendencies are briefly described in the present study.

The availability of phosphorus can be determined by digestibility experiments as outlined by GUEGUEN (1965), by the Göttingen-transponierings-test from GUNTHER and TEKIN (1968) or by the " percentage bone ash " criterium (English literature). The latter method, generally used in poultry experiments, is based on a (linear) relationship between bone ash (tibia) and the percentage of supplemental phosphorus (expressed or not in logarithms), in so far the P-level remains inferior to the P-requirement. Nevertheless, differences in relative biological value (R.B.V.), mentioned in the literature, are due to several factors, which influence the P-utilization (mineral anabolism): basal diet (GILLIS, EDWARDS and YOUNG, 1962; PENSACK and STOCKSTADT, 1961; FRITZ *et al.*, 1969), animal (GILLIS, EDWARDS and YOUNG, 1962), vitamine D_3-level (FRITZ *et al.*, 1969), Ca/P-relationship (WALDROUP, AMMERMAN and HARMS, 1965; DAMRON and HARMS, 1970), Na/Cl-ratio (MONGIN, 1968; BARZEL, 1969; NEWELL and BEAUCHENE, 1975), length of trial period (AMMERMAN *et al.*, 1961; DILWORTH and DAY, 1964; NELSON and PEELER, 1961). The calculation of the R.B.V. is usually based on the three following techniques: the abcissa-method (GILLIS, NORRIS and HEUSER, 1948), the ordinate-method (BARUAH *et al.*, 1960) and the slope ratio-method (HURWITZ, 1964; YOSHIDA and HOSHII, 1977). SULLIVAN (1966) however, combined the three criteria (percent bone ash, weight gain and feed conversion) in a multifactorial regression equation for the calculation of the relative biological utilization values.

This brief survey of literature on the subject shows that a great number of nutritional and physiological factors influence P-utilization by poultry. This, together with some attendant problems with respect to the methodology used explains why the authors disagree on the extent to which the various P-sources are biologically utilized.

Bearing this in mind, the objective was to determine the relative biological utilization of P in various feed phosphates by means of two trials in vivo with broiler chickens.

This investigation was limited to the main feed phosphates, from which the formulator and or the computor must practically make a choice on the basis of their composition (P, Ca, Na, F), the biological availability of P and the price. This investigation comprised: mono-calcium and dicalcium phosphates (hydrous and anhydrous), monosodium- and disodium-phosphates, defluorinated phosphates (DFP), a Ca-Mg-Na phosphate and meat-and-bone meal, all of which are sources of phosphorus with a rather good P-utilization. In order to make interpretation of the results easier, a Ca-Al-Fe-phosphate, which has a poor P-availability for chicks, was included in the outline of the second experiment. The criteria investigated in these experiments were: weight gain, feed conversion, bone ash deposition, percent bone ash, P-retention in the bones and the breaking strength of the tibia. The most important experimental variable studied was the effect of the Ca: P-ratio and of the Na: Cl-ratio.

Materials and methods

A. — *General considerations*

In order to be able to compare the effect of the various P-sources the following conditions must be fulfilled:

1. uniform test groups having a virtually identical mean and distribution of body weight at the beginning of the test period;
2. an equal feed intake per animal-day (pair feeding system), so, provided that there are no interactions with the Ca-level, the contribution of organic P is the same for all test groups (WATKINS and MITCHELL, 1936). Thus, growth improvement occuring at increasing P-levels can only be attributed to a better feed conversion (NELSON and MILES, 1972 and 1973);
3. the P (whole body): P (tibia)-ratio must be almost constant for all P-sources and for all P-supplementations, so that it is sufficient to determine the composition of the tibia. Nevertheless, this ratio depends on age, on the composition of the ration and on environmental factors. In this way the determination of the tibia composition provides a measure for the whole P-retention and, to some extent, for the P-absorption, in so far as the excretion of phosphorus through the urine may be considered to be constant.

B. — *Animals and housing*

The day-old chicks were housed in battery cages. During the first week the broilers were fed ad-libitum a starter ration with an optimal P-level. The trial period started at the age of one week and lasted for 3 weeks, in principle. During this period the chick is subject to a very intense mineral anabolism, resulting in extremely high feed requirements (GUNTHER and TEKIN, 1968). When they were one week old the chicks (males: Ross— 1st trial, Hybro — 2nd trial) were individually weighed and grouped in classes of weight (interval of weight: 10 gram). About 75 p. 100 of the chicks belonging to the medium weight classes were grouped uniformly in test groups of 10 chicks each. There were 12 animals per m^2. During the experimental period, the feed intake was controlled in such a way that the average feed intake was the same for all experimental groups during the whole period. Because of many difficulties it was impossible to obtain an equal daily feed intake for all groups. When the chicks were 4 weeks old, they were weighed by group and killed. The surrounding meat and the cartilage was removed from the tibia's after they had been boiled for 5 minutes. Firstly, the breaking strength of the tibia's was determined: strength meter, Mark; distance between the points of support, 5 cm in the first trial and 3.5 cm in the second trial (shorter tibia's); unit of force: kilogram. Then the dry matter content was determined (16 hours, 65 °C) without any previous defatting of the tibia's. The ash-content was determined by means of an incineration at 580 °C for 10 hours. The P-level of the ash was determined with a double way spectrophotometer (430 *nm*) (phospho-vanado-molybdate of ammonium). The extent to which the P-body: P-tibia ratio was independent of the P-level of the ration was verified only in the first trial. These determinations were carried out in the presence of one P-source.

C. — *The composition of the rations*

Table 1 shows the composition of the sources of phosphorus used for the investigation and table 2 mentions both the composition of the basic rations and the Ca-and P-levels of the various supplements for both experiments. This indicates the composition of the basic ration had to be adjusted to the nature of the P-source. The rest p. 100 of the diet comprises: a Ca-source ($CaCo_3$, $CaCl_2.2H_2o$), a Na-source ($NaCl/NaHCO_3$, Na_2SO_4), a P-source and a supplementary material (to fulfill the 100 p. 100, milo). When calculating the P-level or the metabolizable energy-content (M. E.-content) this supplementary milo was not taken into account. But, it is to be remarked that at the lowest P-supplementation the M.E.-content is about 2 p. 100 higher than at the highest P-supplementation. In order to avoid possible effects on live weight gain and feed conversion the total quantity of soybean meal + balanced meat-and-bone meal (+ amino acids) was maintained at a constant level. For these test groups the variable supply of P through soybean meal was taken into account.

In the 1st experiment there were 8 P-supplementations (1 replicate); in the 2nd experiment there were 4 supplements (2 replicates). Test groups without mineral P-supplementations (basal rations) were included only in the 2nd experiment. Phosphorus was supplied only by the feed, since the broilers received

TABLE I

Composition of the tested P-sources
Composition des phosphates analysés

P-sources	Ca (%)	P-total (%)	% P soluble in (*) citrate (2 %)	Na (%)
1. Monocalcium phosphate : MCP ≠ A	16.9	23.06	99	0.28
2. Monocalcium phosphate : MCP ≠ B	16.83	23.15	99	0.072
3. Hydrated dicalcium phosphate : DCP ≠ A	25.65	17.93	98	0.011
4. Hydrated dicalcium phosphate : DCP ≠ B	27.32	20.48	92	0.039
5. Anhydrous dicalcium phosphate : An DCP ≠ A	29.17	21.38	98	0.028
6. Anhydrous dicalcium phosphate : An DCP ≠ B	29.73	21.16	97	0.029
7. Defluorinated rock phosphate : DFP ≠ A	31.81	18.50	47.6	5.62
8. Defluorinated rock phosphate : DFP ≠ B	31.81	18.11	67.1	4.94
9. Ca-Mg-Na-phosphate : CMP	9.93	17.34	84	11.55
10. Di-sodiumphosphate : DSP	sp	21.26	100	28.74
11. Meat-and-bone meal: MBM	12.09	5.80	—	—
12. Mono-sodiumphosphate: MSP	sp	19.8	100	14.7
13. Ca-Al-Fe phosphate (Al: 19.3 %, Fe: 6.4 %, F: 0.95 %): CAP	7.5	14.5	—	0.6

(*) P. 100 P soluble in citrate (2 p. 100) : agitation in a solution of 2 p. 100 Ammoniumcitrate during 60 min at 20 °C.

TABLE 2

Composition of the basal rations and the experimental diets
Composition des rations de base et des rations expérimentales

A. *Composition of raw materials*

	Basal ration A(2)	Basal ration B (3)
Soybean oil meal (50), % (1)	34.14	29.31
Milo, % (1)	53.91	53.91
Animal fat, % (1)	7.42	7.42
Min.-and vit.-mixture, % (1) (a) . . .	1.00	1.00
Dl-methionine, % (1)	0.23	0.23
Rest, % (*variable composition*)	*3.3*	*8.13*
TOTAL, %.	100.00	100.00
Ca-content, % (1)	0.44	0.42
P-content % (1)	0.38	0.34
Non-phytic, P-content, % (1)	0.143	0.13

(1) Basal part, constant at each P-level.
(2) Basal part for the 1st expriment (P-sources : 1-10 + 12) and for the 2nd experiment.
(3) Basal part for the 1st experiment (P-sources : 11).

B. *The Ca- and P-levels at the different P-supplementations* (*1st experiment*)

	A	B	C	D	E	F	G	H
Total Ca % . . .	0.602	0.675	0.752	0.832	0.916	1.00	1.09	1.19
Total P %	0.415	0.450	0.485	0.520	0.555	0.59	0.625	0.660
Ca/P-ratio	1.45	1.50	1.55	1.60	1.65	1.70	1.75	1.80
Non-phytic P % ( )								
P-source (1 — 10 + 12)	0.178	0.213	0.248	0.283	0.318	0.353	0.388	0.423
P-source 11. . . .	0.219	0.253	0.286	0.319	0.352	0.385	0.418	0.453

C. *The Ca- and P-levels at the different P-supplementations* (*2nd experiment*)

	Basis (BA)	A′	B′	C′	D′
Total Ca %	1.00	1.00	1.00	1.00	1.00
Total P %.	0.380	0.435	0.490	0.545	0.600
Ca/P-ratio.	2.63	2.30	2.04	1.83	1.67
Non-phytic P % (b) .	0.143	0.198	0.253	0.308	0.363

(a) The vitamine D_3-level was about 2 700 I.U. per kg complete diet.
(b) However the non-phytic P-content of the rations can be divided in two parts : firstly the biologically available plant phosphorus (Nelson, 1967) and secondly the added « inorganic » phosphorus.

deionized water (ion exchanger). The Ca: P-ratio did not remain constant when the P-level increased. The Ca: P-ratio increased with increasing P-levels (1st experiment). This method yields higher percentages of bone ash (WALDROUP, AMMERMAN and HARMS, 1965) than the use of a constant Ca-level or a constant Ca: P-ratio. In the 2nd experiment the Ca-level was maintained constant, whereas the P-level increased. This method results in a greater increase of the percent bone ash, mainly since the Ca: P-ratio is too great at the lower P-levels. The Na: Cl-ratio has a considerable effect on the acid-base balance of the body and, hence, also on the live weight gain and the process of mineralization. The optimal Na: Cl-ratio nearly equals 1 and is independent of the Na-level and the Cl-level of the ration (HURWITZ *et al.*, 1973). In the first experiment, the Na- or the Cl-level was 0.175 p. 100 for most of the test groups, except for the groups with P-sources that were rich in sodium. In the second experiment the minimum Na- or Cl-level was 0.15 p. 100 (table 3). Also, in the second experiment 3 Na- Cl ratio's were applied to one source of phosphorus (DCP ≠ A) : Na :Cl = 3:1 (with either HCO_3^- or SO_4^{-2} as a anion), Na: Cl = 1 and Na: Cl = 1:3. However when calculating the linear regression equations, the basal ration taken into account was a ration with a Na: Cl-ratio equal to 1.

TABLE 3

Survey of the experimental designs (1st and 2nd experiment) : the P-sources with different Na /Cl-ratios

Revue du schéma expérimental (1re et 2e expérimentation) : sources de phosphore et différents rapports de Na /Cl

P-sources	1st experiment (increasing Ca /P-ratio) 8 P-supplementations	2nd experiment (constant Ca-level or decreasing Ca /P-ratio) 4 P-supplementations
MCP ≠ A	Na:Cl = 1 (0.175 %)	—
MCP ≠ B	idem	Na:Cl = 1 (0.15 %)
DCP ≠ A	idem	Na:Cl = 1
	—	Na:Cl = 3 /1-HCO_3^-
	—	Na:Cl = 3 /1-SO_4^{--}
	—	Na:Cl = 1 /3
DCP ≠ B	idem	Na:Cl = 1
AnDCP ≠ A	idem	—
AnDCP ≠ B	idem	Na:Cl = 1
DFP ≠ A	idem	—
DFP ≠ B	idem	Na:Cl = 1
CMP	idem	Na:Cl = 1
DSP	idem	Na:Cl = 1
MBM	idem	—
MSP	idem	—
CAP	—	Na:Cl = 1

D. — *Calculations*

For the calculation of the linear regression equations the level of the non-phytic phosphorus (1st experiment) or the level of added " inorganic " phosphorus (2nd experiment) was chosen as the independent variable for the four tibia parameters. In the first experiment it was necessary to do so as the two basal rations used had a totally different P-level. As in the second experiment the birds were fed a basal ration that contained no supplemental inorganic P, we were able to use the corresponding data as a fixed point on the x-y diagram that was common to all of the calculated linear regression equations. For reasons further explained in the section headed " Results and Discussion ", the area under the linear regression lines was considered as a criterion of the biological utilization of phosphorus in the 1st experiment, whereas in the second experiment the slopes of the regression lines were used as a criterion of the R.B.V.

Results and discussion

The results of the investigated parameters, obtained at each P-levels, are not mentioned to avoid no surveyable tables. On the other hand, these results are taken into account for the calculations of the linear regression equation in so far there was a linear relationship between the P-level and the above mentioned parameters (Tables 4 and 5). Table 6 summarizes the results regarding the R.B.V.

A. — *Effect of the P-level (Ca-level)*

1. *First experiment*

The application of a controlled feeding technique enabled the maintenance of an average feed intake, for the whole experimental period, at the same level for all experimental groups: about 1 335 g/animal. The importance of this factor was indicated in the section " Trial Methods ". The phosphorus consumed by the chicks is both organic and inorganic. At an equal food intake the share of the qualitatively unknown organic P may be considered to be constant. Thus any effect on the feed efficiency on the body weight and on the tibia-parameters can only be due to a variable quantity of supplemented (available) inorganic P. An increase of the inorganic P-level (and of the Ca-level in the first experiment) had a favourable effect on both the live weight gain and the feed conversion. Moreover, all the calculated features of the tibia (breaking strength, ash-content, ash-percentage and P-level) continuously improved. Under the prevailing experimental conditions the P (body): P (tibia) — ratio amounted to 17.95 ± 0.46 on average, with the P-level of the ration being < 0.6 p. 100. HURWITZ (1964) obtained a ratio of 19.6 ± 1. At higher P-levels the ratio amounted to 16.8 ± 0.81. This points to a relative gain of P in the skeleton, c.q. in the tibia. Consequently, we may assume the P-level of the tibia as a good indicator of the amount of total phosphorus retained, when the p. 100 of P in the ration is $<$ 0.60 p. 100. There is a linear relationship between the above mentioned parameters and the P-level of the ration, as long as a particular P-level is not exceeded. For body weight gain, feed conversion, ash-content and P-level parameters, this particular P-level is 0.60 p. 100 and for the tibia-ash percentage parameter it amounts to 0.66 p. 100. So, it is possible, with respect to this last parameter to carry out measurements in a greater linear area. When calculating the linear regression equations the values

TABLEAU 4

The linear regression equations: the effect of the non-phytic P-content on body weight, feed conversion, tibia-breaking strength, tibia-ash content tibia-ash percentage and tibia-P-content (1st experiment) (standard deviation on y = Sy; correlation coefficient, r^2)

Équations linéaires de regression: effet de la teneur en P non-phytique sur le poids corporel, l'indice de consommation, la résistance à la fracture osseuse, la teneur en cendres du tibia, le pourcentage de cendres du tibia et la teneur en P du tibia (1re experimentation) (l'écart- type sur Y = Sy; coefficient de correlation, r^2)

P-sources	Body weight (g)			Feed conversion (kg/kg)			Breaking strength (kg)		
	Linear regression equation	Sy	r^2	Linear regression equation	Sy	r^2	Linear regression equation	Sy	r^2
MCP ≠ A	$y = 136x + 819$	14,2	0,53	$y = -0{,}235x + 1{,}91$	0,030	0,69	$y = 19{,}1x + 5{,}51$	1,31	0,90
MCP ≠ B	$= 334x + 743$	25,6	0,97	$= -0{,}908x + 2{,}14$	0,030	0,91	$= 35{,}4x + 0{,}73$	2,42	0,91
DCP ≠ A	$= 493x + 711$	43,3	0,89	$= -1{,}214x + 2{,}21$	0,040	0,92	$= 33{,}3x + 1{,}83$	2,21	0,97
DCP ≠ B	$= 351x + 760$	27,2	0,91	$= -0{,}694x + 2{,}02$	0,021	0,86	$= 48{,}0x - 2{,}31$	3,24	0,93
An-DCP ≠ A	$= 500x + 716$	39,9	0,91	$= -1{,}316x + 2{,}22$	0,101	0,88	$= 47{,}1x - 2{,}61$	3,16	0,95
An-DCP ≠ B	$= 338x + 756$	31,9	0,98	$= -0{,}796x + 2{,}08$	0,064	0,87	$= 37{,}6x - 0{,}30$	2,61	0,88
DFP ≠ A	$= 274x + 763$	21,8	0,93	$= -0{,}765x + 2{,}10$	0,059	0,94	$= 49{,}2x - 2{,}05$	3,24	0,99
DFP ≠ B	$= 495x + 714$	51,4	0,96	$= -1{,}316x + 2{,}22$	0,112	0,78	$= 44{,}6x - 0{,}98$	3,05	0,88
CMP	$= 718x + 479$	37,8	0,74	$= -1{,}222x + 2{,}17$	0,091	0,88	$= 40{,}3x - 0{,}19$	2,64	0,95
DSP	$= 784x + 387$	34,3	0,88	$= -0{,}908x + 2{,}14$	0,075	0,84	$= 40{,}6x - 0{,}11$	2,70	0,94
MBM	$= 310x + 766$	24,0	0,86	$= -0{,}742x + 2{,}06$	0,059	0,85	$= 43{,}4x - 1{,}51$	2,52	0,92
MSP	$= 193x + 784$	27,6	0,89	$= -0{,}592x + 2{,}05$	0,051	0,75	$= 40{,}5x - 0{,}29$	2,74	0,94
	Ash content (mg)			Ash-percentage (%)			P-content (mg)		
	Linear regression equation	Sy	r^2	Linear regression equation	Sy	r^2	Linear regression equation	Sy	r^2
MCP ≠ A	$y = 1\,724x + 476$	113	0,99	$y = 24{,}7x + 34{,}4$	1,91	0,94	$y = 358x + 80{,}8$	23,7	0,98
MCP ≠ B	$= 2\,220x + 319$	147	0,97	$= 32{,}8x + 31{,}8$	2,51	0,98	$= 464x + 45{,}4$	31,0	0,98
DCP ≠ A	$= 2\,192x + 365$	145	0,98	$= 32{,}8x + 32{,}4$	2,47	0,95	$= 384x + 71{,}7$	25,3	0,98
DCP ≠ B	$= 2\,369x + 262$	156	0,99	$= 37{,}1x + 30{,}1$	2,83	0,99	$= 451x + 49{,}2$	29,3	0,97
An-DCP ≠ A	$= 2\,423x + 260$	165	0,99	$= 32{,}9x + 31{,}5$	2,47	0,96	$= 477x + 40{,}8$	32,2	0,98
An-DCP ≠ B	$= 1\,824x + 408$	120	0,99	$= 26{,}2x + 33{,}5$	2,09	0,92	$= 385x + 65{,}2$	25,6	0,98
DFP ≠ A	$= 2\,475x + 265$	164	0,97	$= 35{,}6x + 31{,}4$	2,76	0,92	$= 424x + 58{,}0$	28,1	0,98
DFP ≠ B	$= 2\,342x + 316$	155	0,97	$= 36{,}2x + 31{,}3$	2.75	0,98	$= 424x + 55{,}7$	11,5	0,98
CMP	$= 2\,309x + 336$	153	0,97	$= 27{,}1x + 34{,}5$	2,16	0,90	$= 305x + 92{,}9$	21,4	0,90
DSP	$= 2\,335x + 308$	152	0,98	$= 33{,}3x + 32{,}8$	2,55	0,96	$= 467x + 49{,}9$	31,9	0,98
MBM	$= 2\,594x + 231$	142	0,97	$= 37{,}2x + 30{,}7$	2,41	0,96	$= 385x + 66{,}6$	21,2	0,95
MSP	$= 2\,098x + 381$	144	0,91	$= 32{,}8x + 32{,}4$	2,55	0,94	$= 424x + 59{,}1$	28,3	0,96

TABLE 5

The linear regression equations for the tibia-properties: the effect of non-phytic P-content on breaking strength, ash-content, ash-percentage and P-content (2nd experiment).
(standard deviation on y = Sy; determination coefficient, r^2)

Équations linéaires de regression des paramètres du tibia: effet de la teneur de P-non-phytique sur la résistance à la fracture osseuse, teneur en cendres, pourcentage de cendres et teneur en P (2[e] épreuve)
(L'écart-type de y = Sy; coefficient de détermination, r^2)

P-sources	Ash-content			Breaking strength (kg)			Ash-percentage — (%)			P-content (mg)		
	Linear regression equation	Sy	r^2	Linear regression equation	Sy	r^2	Linear regression equation	sy	r^2	Linear regression equation	Sy	r^2
	(**)			(**)			(**)			(**)		
MCP ≠ B	y = 1 392x (*)	30	0,98	y = 46,7x (*)	0,96	0,98	y = 29,7x (*)	0,20	0,99	y = 285x (*)	4,9	0,98
DCP ≠ B	= 1 436x	19	0,99	= 47,2x	1,66	0,94	= 28,3x	0,29	0,99	= 272x	6,1	0,98
DCP ≠ A	= 1 535x	31	0,98	= 51,7x	1,44	0,96	= 31,7x	0,18	0,99	= 281x	8,2	0,96
DCP ≠ A (Na/Cl = 3/1 HCO_3^-) .	= 1 579x	20	0,99	= 49,7x	1,8	0,93	= 34,7x	0,29	0,98	= 305x	3,6	0,99
DCP ≠ A (Na/Cl = 1/3)	= 1 311x	19	0,97	= 42,3x	1,3	0,97	= 27,4x	0,25	0,99	= 264x	3,9	0,98
DCP ≠ A (Na/Cl = 3/1 SO_4^{-2}). .	= 1 200x	31	0,98	= 40,9x	1,9	0,93	= 28,6x	0,06	0,99	= 248x	2,4	0,99
An DCP ≠ B. . .	= 1 205x	8	0,99	= 43,4x	0,78	0,98	= 23,1x	0,27	0,99	= 228x	2,4	0,99
DFP ≠ B	= 1 311x	31	0,98	= 45,6x	1,10	0,97	= 28,9x	0,21	0,99	= 282x	4,1	0,99
CMP	= 1 541x	44	0,95	= 48,3x	1,9	0,93	= 34,8x	0,23	0,99	= 289x	6,2	0,98
DSP	= 1 427x	22	0,99	= 50,6x	2,0	0,93	= 31,3x	0,32	0,98	= 287x	8,3	0,96
CAP	= 155x	24	0,99	= 9,9x	0,9	0,96	= 4,8x	0,25	0,91	= 32x	0,82	0,97

(*) x = X — 0,143 with X = the non-phytic P — p. 100.
(**) The common intercept values (x = 0) omitted : These are 6 (breaking strength), 470 (ash-content), 37 (ash-percentage) and 83 (P-content).
Les valeurs ordonnées communes à l'origine (x = 0) sont supprimées : elles sont 6 (fracture osseuse), 470 (teneur en cendres), 37 (pourcentage de cendres) et 83 (teneur en P).

TABLE 6

A general view of the relative biological values, obtained from the 1st and 2nd experiments

Vue générale des valeurs biologiques relatives, obtenues de la 1re et 2e expérience

P-sources	Na:Cl ratio	1st experiment (area)					2nd experiment (slope)				
		Ash-%	Ash-content	P-content	Breaking strength	Average	Ash-%	Ash content	P-content	Breaking strength	Average
MCP ≠ A. .	1:1	89	101	103	98	98	—	—	—	—	—
MCP ≠ B. .	1:1	85	92	90	90	89	95	97	103	92	97
DCP ≠ A. .	1:1	91	105	99	100	99	101	108	102	102	103
	3:1-HCO_3^-	—	—	—	—	—	111	111	105	98	106
	3:1-SO_4^-	—	—	—	—	—	91	84	94	84	88
	1:3	—	—	—	—	—	87	92	91	81	88
DCP ≠ B . .	1:1	84	89	93	96	90	90	101	98	93	95
AnDCP ≠ A	1:1	81	89	89	87	86	—	—	—	—	—
AnDCP ≠ B	1:1	82	85	89	83	85	84	84	89	86	86
DFP ≠ A. .	1:1	90	97	93	106	96	—	—	—	—	—
DFP ≠ B. .	1:1	91	102	93	104	96	92	92	100	90	94
CMP	1:1	99	106	100	98	101	111	108	103	95	104
DSP	1:1	*100*	*100*	*100*	*100*	*100*	*100*	*100*	*100*	*100*	*100*
MBM. . . .	1:1	86	95	90	88	90	—	—	—	—	—
MSP	1:1	92	102	95	96	96	—	—	—	—	—
CAP	1:1	—	—	—	—	—	15	11	13	20	15
LSD (*). . .		—	—	—	—	—	25	29	26	37	—

(*) Least significant difference (2nd experiment); p : 0.05.

corresponding to P-levels above the already mentioned limits, were not taken into account. The linear relationship between the P-level of the ration or the P-intake by the chicks, on the one hand, and the parameters labeled ash-content, ash-percentage and P-level of the tibia on the other hand, may be qualified as " very good " for all the sources of phosphorus examined, as it is shown by the determination coefficients (r^2) and the standard deviation (Sy) mentioned in table 4 and by figures 1, 2 and 3. This also holds, be it to a lesser extent, for the breaking strength. There are also high correlation coefficients between the various tibia-parameters above: 0.89 — 0.98 ($n = 96$). The lowest correlation coefficients relate to the " breaking strength " parameter. On the other hand, the relationship with the parameters " live weight gain " and " feed conversion " is clearly a non-linear and less accurate one, so that these parameters are not taken into account when determining the R.B.V.

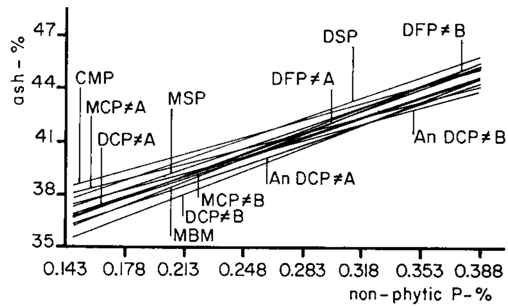

FIG. 1. — *The effect on non-phytic P-content on tibia ash percentage* (1st experiment).
Effet de la teneur en P non-phytique sur le pourcentage des cendres du tibia (1re épreuve).

2. *Second experiment*

The feed intake was considerably lower than in the first experiment and amount on average to 854 g /animal for all test groups. In this experiment, both the body weight and the feed conversion were virtually independent of the P-level of the ration (fig. 2-3). Similar results were also obtained by HURWITZ (1964). In this experiment the average final weight was 577 g and the average feed conversion was ± 1.73.

Although there is no obvious explanation for this phenomenon, particular attention should be paid to the factors that, compared to the 1st experiment, were different, namely a constant Ca-level or a declining Ca: P-ratio and a low live weight gain, resulting from the lower feed intake (pair feeding system).

Consequently, an increase of the P-level only resulted in a considerable improvement of the features of the tibia. The linear relationship between the features of the tibia and the P-level covers a smaller P-supplementation area than in the previous experiment. Consequently, the upper limits of the various tibia-parameters are slightly lower than in the first experiment: 0.54 p. 100 P for the breaking strength, the ash-content and the P-level and 0.57 p. 100 for the ash-percentage respectively. On the basis of the non-phytic P-level of the ration (fig. 2) especially the ash-content and the P-level of the tibia are considerably lower than in the first experiment, whereas the breaking strength and the ash-

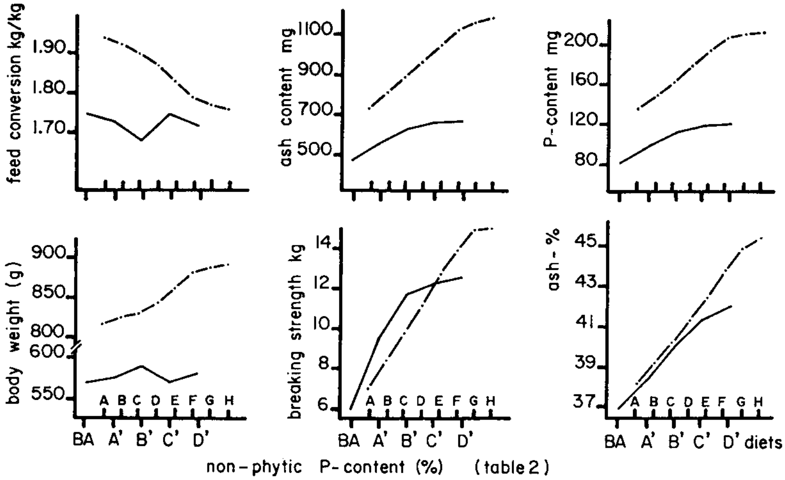

FIG. 2. — *A comparison of the results, obtained from the 1st and 2nd experiment* (average from all P-sources).

Comparaison des résultats, obtenus de la 1re et de la 2e épreuve

.—.—.—. 1st experiment
———— 2nd experiment

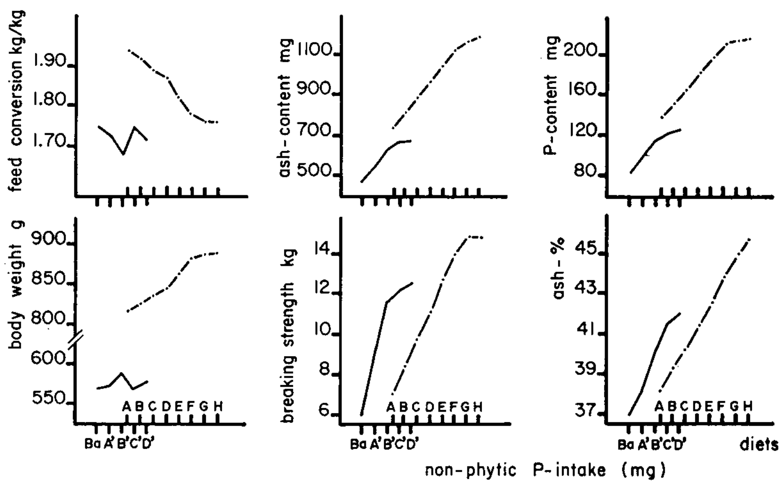

FIG. 3. — *A comparison of the results, obtained from the 1st and 2nd experiment* (average for all P-sources).

Comparaison des résultats, obtenus de la 1re et 2e épreuve (moyenne de toutes les sources de P).

.—.—.—. 1st experiment
———— 2nd experiment

percentage of the tibia differ only slightly from the analogous observations made in the first experiment. With respect to the breaking strength attention should however be paid to the difference of the distance between the points of support used when determining the breaking strength, so that it is difficult to compare these observations. As for the ash-percentages it needs to be mentioned that this is in fact a ratio of two absolute values, i.e. the ash content (mg) and the total amount of dry matter of the tibia. Absolute values seemed to be more considerably influenced by breed, age, and feed intake than relative values (percentages). If the observations are expressed in terms of intake of non-phytic phosphorus (fig. 3), the response of ash deposition (ash content, mg) and P-retention (P-level, mg) to increasing P-supplements appears to be nearly parallel in both experiments. The ash-percentage, on the contrary, is clearly higher in the second trial, which is probably explained by the lower tibia-bone weights.

B. — *Effect of the nature of the sources of phosphorus*

More markedly than in the first experiment, linear response is missing between, on the one hand, the parameters " body weight — feed conversion " and the P-level of the ration on the other hand. The linear relationship between the

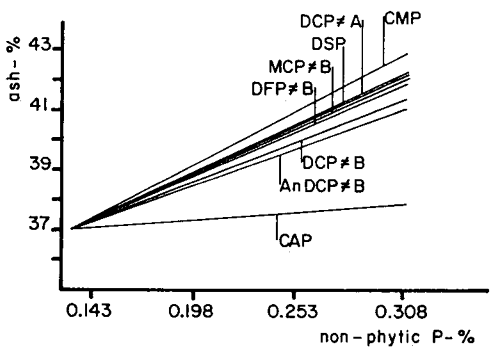

FIG. 4. — *The effect of non-phytic P-content on tibia ash-percentage* (2nd experiment).
Effet de la teneur en P non-phytique sur le pourcentage des cendres du tibia (2^{e} épreuve).

tibia-parameters and the P-level of the ration was, however, very good for all P-sources involved in the examination (Tables 4 and 5, fig. 1 and 4). It is for this reason as for the first experiment, that the parameters " body weight — feed conversion " were not taken into account when calculating the relative biological values (R.B.V.). Consequently, the R.B.V. are in both experiments based on the tibia-parameters. As, in a previously established P-supplement area ($\leqslant$ optimal P), there is a linear relationship between the tibia-parameters and the P-level of the ration, it is sufficient to compare the linear regression equations to each other. Different techniques have been used to this end:

1. The ordinate-method (BARUAH *et al.*, 1960): at a previously fixed abscissa value the y-values are compared to each other. The P-source the regression of which yields the greatest y-value at one particular x-value is considered to be the best biological source.
2. The slope-method (HURWITZ, 1964): the slopes of the linear equations were compared to each other. The P-source the regression of which has the greatest slope, presents the best P-utilization.

3. The abscissa-method (GILLIS, NORRIS and HEUSER, 1948): comparison of the abscissa's corresponding to a previously chosen ordinate. The P-source the regression equation of which has the lowest x-value, at one single y-value is the best biological source.

These methods may, or may not, lead to contradictory conclusions, when evaluating the R.B.V. In fact these contradictions are due to the situation of the linear regression lines (convergent-parallel-divergent) and to the (absolute) values necessary for the calculation of the relative biological values. If the lines are

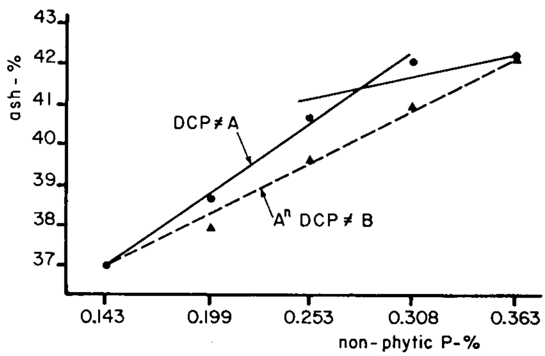

FIG. 5. — *The effect of non-phytic P-content on tibia ash-percentage* (2nd experiment: divergent-convergent).

Effet de la teneur en P non-phytique sur le pourcentage des cendres du tibia (2^e^ *épreuve* : divergent-convergent).

divergent (2nd experiment) the R.B.V.'s are independent of the interpretation technique and of the P-level of the ration. When the lines are convergent or parallel (1st experiment) the R.B.V.'s are dependent of both the interpretation technique and the P-level of the ration. The order of the R.B.V.-classification obtained with the slope-method (convergent lines) is the opposite of the one obtained with the abscissa method, which leads to an exact evaluation. The choice of ordinates and abscissa's influences the R.B.V. in the case of convergent and parallel lines. So, it is necessary to calculate the average biological availability for the whole linear area. But, in order to avoid this complicated calculation, the area under the regression lines may be considered to be a representative estimate of the R.B.V. for the whole area of linear response to P-supplementation. It is striking that the experimental circumstances, e.g. the feed intake and the composition of the rations (the Ca: P-ratio), clearly influence the total picture of the regression lines convergent-parallel (1st experiment) and divergent (2nd experiment). In the first experiment the Ca: P-balance was however more optimal than in the second experiment. If we consider the whole area of P-supplementation, high-grade sources of phosphorus show a rather asymptotic or logarithmic picture (fig. 5-2nd experiment), whereas the graphic of the less available sources is virtually linear. At increasing P-levels of the rations the originally divergent lines, change into parallel lines and finally show a convergent picture. So, the utilization of absorbed phosphorus derived from high grade sources of phosphorus is better when the P-supplementation is low. If the P-levels of the ration increase and the absorption coefficient remains constant, the mineral retention (ash and P in the tibia) will more markedly decline in the case of these sources than in the case of sources having a lower biological value. Consequently, it may be inferred that in the case of high-grade sources the physiological ash- and P-saturation of the skeleton (tibia) will occur sooner, when the dietary P-levels increase, so that the P-excretion in the urine will have a considerable effect upon the P-utilization. Table 6, which summarizes the R.B.V. of the P-sources examined here, proves that the various P-sources do not show very important differences in R.B.V. (maximum 15-20 p. 100), except for the Ca-Al-Fe-phosphate, the R.B.V. of which is very low and amounts to only 15 p. 100 of the R.B.V. of disodium phosphate.

The results of the 1st and of the 2nd experiment are very much alike (differences of 1-8 p. 100), which means that the experimental circumstances, the Ca: P-ratio e.g., did virtually not influence the R.B.V. On the other hand, differences in R.B.V. occurred, according to which tibia-parameters had been chosen.

In this experiment there was not a close relationship between the NH_4^+-citrate solubility and the R.B.V.: the examined DFP with a low solubility have a rather high R.B.V., whereas the anhydrous DCP have a lower R.B.V., in spite of their greater solubility. This means that there are, in addition to the NH_4^+-citrate solubility, other factors, e.g. crystalline and chemical structure and impurities that have an effect upon the absorption and the utilization of the dietary phosphorus. On the other hand, the solubility in diluted citric acid solution — 0.5 p. 100, YOSHIDA *et al.* (1979) and 2 p. 100, GUEGUEN (1970) — can successfully be used as an index of biological availability.

Relative important differences in R.B.V. may occur between some analogous sources of phosphorus, e.g. ± 9 p. 100 between the two MCP and the two DCP, while there are virtually no differences for other analogous sources of phosphorus, in this case the anhydrous DCP and DFP.

This fact may be related to the manufacturing technique and to the attendant features of crystals; the important cause of the R.B.V. decrease is the presence of metaphosphate- and pyrophosphate- radicals, the level of which increases at higher manufacturing temperatures (MC GILLIVRAY, 1978). According to DAMRON and HARMS (1970) important R.B.V.-differences may occur between analogous sources of phosphorus (DFP, rock phosphates), whereas the R.B.V. of DCP appears to be some 12 p. 100 lower than the R.B.V. of monosodium phosphate. The lower R.B.V. of anhydrous DCP, as compared to the R.B.V. of DCP-hydrated, appears clearly from our results, and are in agreement with those obtained by GILLIS, EDWARDS and YOUNG (1962). Moreover, these authors claim the R.B.V. of MCP is markedly (± 15 p. 100) better then the R.B.V. of DCP, which was not so apparent in our experiments. In general, conclusions drawn from the herein reported experiments, concerning the relative biological availability of P in different feed grade phosphates, are in agreement with the U.S.A. literature, in showing: (on poultry)

(1) that there can exist differences in R.B.V. of some 10-15 p. 100 between different sources of commercial DCP, of commercial MCP and of mixtures thereof (NELSON and WALKER, 1964; PENSACK, 1974, MC GILLIVRAY, 1978).

(2) that anhydrous DCP is at least 15 p. 100 lower in R.B.V. than the dihydrate form of DCP (GILLIS, EDWARDS and YOUNG, 1962).

The generally observed higher biological availability of 5-15 p. 100 in monocalcium phosphates and in mono-disodium phosphates compared to dicalcium-phosphate (GILLIS, EDWARDS and YOUNG, 1962; NELSON and WALKER, 1964;

PENSACK, 1974; DAY, MC NAUGHTON and DILWORTH, 1973; DAMRON and HARMS, 1970; MC GILLIVRAY, 1978) is not confirmed by our experimental results. It demonstrates, in agreement with the work of FRITZ *et al.* (1969), that such difference is not always valid for all sources of these feed grade phosphates.

The studies of BRUNE und GÜNTHER (1961) with rats and GÜNTHER (1966*a*, *b*) and GUNTHER und TEKIN (1968) with rats and chicks have shown that the absence of vitamin D_3 increases considerably the difference in biological efficiency between mono-and di-calcium phosphate, while in the presence of adequate amounts of vitamin D_3 the difference becomes minor or disappears.

The R.B.V. found for the Ca-Mg-Na-phosphate shows that the phosphorus

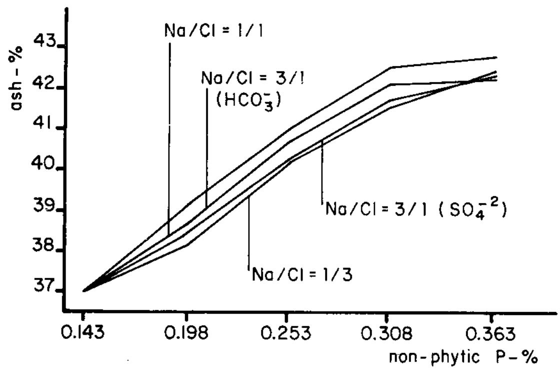

FIG. 6. — *The effect of non-phytic P p. 100 and Na : Cl-ratio, combined with different anions on tibia ash-percentage* (2nd experiment).

Effet de la teneur en P non-phytique et du rapport de Na : Cl, associé de différents anions sur le pourcentage des cendres du tibia (2^e épreuve).

availability in this mixed phosphate is comparable, but not much higher, than that from the best commercial sources of mono-and di-calcium phosphate. This is in agreement with the conclusion, which can be drawn from a comparison of the results of BRUNE und GÜNTHER (1961), STAPPERS, BORGGREVE and GRIMBERGEN (1974) and of the biological efficiency level of 121, found for this Ca-Mg-Na-phosphate (ROSIN, 1972).

The utilization of phosphorus not only depends on the absorption level in the intestinal lumen, but also on the reabsorption level of the renal tubuli. The reabsorption is considerably influenced by the P-level of the blood plasma (physiological saturation) and by the acid-base balance of the plasma (Na: Cl-ratio). The Na: Cl-ratio can only have an effect upon the acid-base balance, if the anion is metabolizable (HCO_3^-/SO_4^{-2}). The SO_4^{-2}-ion may be considered to be partially metabolizable, if it can be used to meet the sulphate requirement of the chick. In the case of a diminishing Na: Cl-ratio, not only NH_3 but also HPO_4^{-2} is being excreted to neutralize the urine (MONGIN, 1968). This results in a decline of the utilization of dietary phosphorus, so that it takes longer to achieve physiological saturation. Figure 6 illustrates this thesis. Table 6 shows that an increase of the Na: Cl-ratio from 1: 1 to 3: 1 with the metabolizable HCO_3^- ion, increases the R.B.V., of DCP $\neq$ A by 3 p. 100 on the basis of the 4 tibia parameters. On the contrary, a narrower Na: Cl-ratio of 1: 3, reduces the utilization of phosphorus derived from DCP $\neq$ A by 15 p. 100, from 103 to 88. An increase of the Na:

Cl-ratio to 3: 1 with the SO_4^{-2}-ion results also in a markedly lower utilization of the phosphorus (— 15 p. 100), which may point to the fact that the SO_4^{-2} is not metabolized, because of an excess of dietary S under the given experimental conditions. The observations concerning the negative influence of acidosis on P-utilization and on the biological availability of P in DCP are in agreement with experimental results from BARZEL (1969) and NEWELL and BEAUCHENE (1975) with rats and from JAMBOR and PROCHAZKA (1977) with pigs. They observed that these acid-stressed animals showed significant increases in urinary total phosphorus and calcium excretions. SAUVEUR *et al.* (1977) have also shown that metabolic acidosis inhibits the renal conversion of 25-(OH)-vit. D into 1,25-$(OH)_2$ vit. D by about 40 p. 100 in rachitic chicks. The metabolically active 1,25-$(OH)_2$-vit. D not only stimulates the renal P-reabsorption, but also the P-transport through the intestinal cell walls (WALLING, 1977). Consequently, this physiological condition due to an excess of anions, leads to a lower P-(Ca-) retention.

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Résumé

Valeur biologique relative des sources alimentaires de phosphore pour les poulets de chair

Les valeurs biologiques relatives (R.B.V.) des sources suivantes de phosphore ont été déterminées : deux phosphates monocalciques (MCP), deux phosphates bicalciques hydratés (DCP), deux phosphates bicalciques anhydres (An DCP), deux phosphates défluorés (DFP), un phosphate de Ca-Mg-Na (CMP), un phosphate-mono-sodique (MSP), un phosphate-bi-sodique (DSP) (phosphate de référence), une farine d'os et de viande et un phosphate de Ca-Al-Fe (CAP). Deux épreuves ont été faites : un rapport croissant de Ca:P (1re épreuve) et une teneur constante de Ca (2e épreuve). L'effet du rapport Na: Cl était également considéré. Seuls les paramètres du tibia (résistance de l'os à la fracture, teneur en cendres, pourcentage de cendres et teneur en P) ont été impliqués dans le calcul des R.B.V.

Les poulets de chair ont été logés dans des batteries et dans des conditions pratiques normales (eau déionisée). Les rations, déficientes en P, ont également été distribuées aux groupes expérimentaux uniformes, pendant une période d'anabolisme intense des minéraux (7-20 jours).

Les résultats des deux épreuves sont très analogues (1-8 p. 100). Les moyennes de R.B.V. des deux épreuves sont, en comparaison avec le DSP (= 100) : MCP $\neq$ A = 98, MCP $\neq$ B = 93, DCP $\neq$ A = 101, DCP $\neq$ B = 92, An DCP $\neq$ A = 86, An DCP $\neq$ 86, DFP $\neq$ A = 96, DFP $\neq$ B = 95, CMP = 102, MSP = 96, MBM = 90 et CAP = 15 p. 100. Des différences nettes de solubilité dans le citrate d'ammonium à 2 p. 100 ne correspondent pas à des différences analogues de R.B.V. L'utilisation (rétention) du P était très nettement favorisée par un rapport élevé de Na: Cl, associé à un anion métabolisable.

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