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Availability of phosphorus from field bean *(Vicia faba)* and lupin *(Lupinus albus)* seeds to broiler chickens

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ABSTRACT

Experiment was conducted on 64 broiler chickens from 0 to 18 days of age to evaluate phosphorus availability from lupin and field bean. As indicators, bone mineralization and breaking strength and the retention of P fractions were used.

Body weight at 18 days of life was reduced ($P < 0.01$) when P deficient semi-synthetic diet without inorganic P (P0) was fed. Femur ash content and tibia breaking strength were lowest and similar in chickens fed P–deficient (P0) and field bean containing (BP0) diets. Their femur ash content and tibia breaking strength were lower by about 12% ($P \leqslant 0.05$) and 45% ($P < 0.01$) than of chickens fed a diet with 0.2% of inorganic P (P2) which showed the best bone mineralization.

Phosphorus was more available from lupin than from field bean (judged from breaking strength measurements: respectively 53 and 0% compared with inorganic P considered as 100% available). The results are discussed with respect to validity of the assays used and anti-nutritive factors in field bean.

KEY WORDS: lupin, field bean, phosphorus, bioavailability, bone, chickens

INTRODUCTION

About 70% of cereal grain and protein seed phosphorus (P) is in the form of phytates, and its bioavailability for monogastrics is not constant (Reddy et al., 1982).

Our earlier experiments with germinated triticale indicated that without inorganic P supplementation and at relatively low dietary calcium levels, the utilization of P from cereal grains and high protein seeds in chickens may be higher than the widely accepted 30%. The digestibility and phytic P retention may be as high as 60–70% (Antoniewicz et al., 1992), similar to values found by Aw-Yong et al. (1983) in short-term total collections. Presumably, lower P levels are required to obtain maximum body weight gain than for the highest bone mineralization, providing that dietary Ca:P ratio lies within 1–1.5 (Günther et al., 1982, Hulan et al., 1985).

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A possible decrease of inorganic P supplementation of poultry diets has become in many countries a very important issue from the point of view of reducing both production costs and environmental pollution with this element. This was the motivation for evaluation of P availability from home-grown leguminous seeds that are potentially valuable feedstuffs for growing chickens.

The study was undertaken to investigate the effect of the inclusion of 20% field bean or lupin with or without inorganic P supplementation to broiler diets on total and phytic P retention, and P availability measured by performance, bone mineralization and breaking strength.

MATERIAL AND METHODS

Sixty four one-day old broiler chickens were kept in individual balance cages and fed experimental diets shown in Table 1. A basal low-phosphorus (0.38 and 0.19% of total and non-phytic P, respectively) semi-synthetic diet containing thermally (60°C) denaturated freeze-dried egg albumen and glucose, fortified

Composition of the diets, g/kg TABLE 1

Ingredients	Diets							
	P0	P1	P2	P3	LP0	LP2	BP0	BP2
Maize (ground)	380	380	380	380	380	380	380	380
Soya bean meal	250	250	250	250	250	250	250	250
Egg albumen[a]	89	89	89	89	0	0	25	25
Glucose	172.4	159.4	146.5	133.5	81.3	54.4	45.8	20
Lupin seed (ground)	0	0	0	0	200	200	0	0
Field bean (ground)	0	0	0	0	0	0	200	200
Plant oil	40	40	40	40	40	40	40	40
$NaH_2PO_4 \cdot H_2O$	0	4.5	8.9	13.4	0	8.9	0	8.9
Cellulose	26	26	26	26	0	0	12	12
Minerals and micro--components[b]	42.6	51.1	59.6	68.1	49.7	66.7	47.2	64.1

[a] Thermally denaturated and freeze-dried

[b] Providing (per 1 kg of diet): Premix DKA-Starter 25 g, NaCl 3 g, methionine – diets P0-P3 2.5 g, LP0, LP2 4.2 g, BP0, BP2 3.5 g, lysine 1 g, choline 1 g, biotin 0.3 mg, limestone to produce Ca: P ratio 1.5

	P0	P1	P2	P3	LP0	LP2	BP0	BP2
From analyses:								
Phosphorus (g/kg)								
total	3.8	5.2	5.8	7.0	5.3	7.0	5.1	7.3
phytic	1.9	1.9	1.9	1.9	2.9	2.9	2.7	2.7
non-phytic	1.9	3.3	3.9	5.1	2.4	4.1	2.4	4.6
Crude protein (g/kg)	220	218	221	217	226	223	222	219
Crude fibre (g/kg)	46	43	46	45	54	54	51	54

with vitamin-mineral premix, choline and biotin was either supplemented with graded levels (0.1–0.3%) of inorganic phosphate as NaH_2PO_4 (respectively diets P1–P3) or with 20% of ground lupin (Lupinus albus) (L) or field bean (Vicia faba) (B). The vegetable protein sources were included to replace egg albumen and glucose, essentially on an isoprotein and isoenergetic basis, with or without 0.2% inorganic P addition (diets LP2, LPO, BP2, BPO, respectively). The fibre content of the diets was equalized by using Whatman cellulose powder. Total Ca to total P ratio was kept constant at 1.5 by varying limestone amount in a mineral supplement (Table 1). The content of total / phytic P in the lupin and field bean seeds respectively was 7.6/5.0 and 6.2/4.1 g/kg.

The experimental diets and deionized water were given ad libitum. The experiment lasted 18 days including a balance trial during the final 7 days. Total feed intake in the whole experiment and the balance period, and initial and final body weights were determined. The chickens were sacrificed by decapitation at 18 days to obtain femur and tibia samples.

Sample preparation and chemical and physico-chemical analyses

Soft tissues were cleaned from the bones after cooking in boiling water for 1 min. Femurs were defatted with diethyl ether, dried overnight at 105°C and ashed at 550°C.

Tibia breaking strength was determined immediately after bone preparation using a home-made equipment. An increasing load was applied at the middle of the bone, with the distance between the points of support of 3.5 cm. The breaking load was considered as breaking strength.

Basal nutrients were determined by standard methods. Phytate P in feeds and faeces was determined by a precipitation procedure, similar to that of Tangkong-chitr et al. (1981). A sample 0.3–0.8 g was extracted with 50 ml 1.2% HCL containing 10% Na_2SO_4 by shaking at room temperature for 2 h, and the mixture centrifuged at 3000 g for 20 min. Phytic acid was precipitated from an aliquot of clear extract by adding an equal volume of 0.4% $FeCl_3 \cdot 6H_20$, and heating in a boiling water bath for 30 min. After cooling in an ice-bath for 15 min, ferric phytate was centrifuged into a pellet at 3000 g for 15 min. Total P in the sediment was determined colorimetrically after wet digestion with sulphuric acid and hydrogen peroxide. Colour was developed with ammonium molybdate following by Fiske-Subbarow reagent (Fiske and Subbarow, 1925) and read at 825 nm using a Vitatron photometer. The same colorimetric method was applied to total P determinations in feed and faeces samples.

Phosphorus availability evaluation

The relative availability of P from the seeds was evaluated based on the relationships between the logarithms of dietary P content (X) and the values of selected traits and measures (Y), and by comparing net effects of P addition from the feeds evaluated and from inorganic P. Calculations were made using the response ratio in relation to the diets containing equivalent amounts of inorganic P. The effect of inclusion of the seeds on the P availability from the whole diet was also determined using the same relationship and method.

Statistical analysis

All results were subjected to the analysis of variance. Significance of differences was stated using F test. Treatment means were compared by Duncan multiple range test. The relationships between the traits were characterized by calculation correlations and simple linear regressions.

RESULTS

Chicken performance and phosphorus utilization parameters are given in Table 2. No increase in mortality was found in the chicks from the group fed P deficient semi-synthetic diet without inorganic P addition. However, the birds grew more slowly than did those consuming diets supplemented with 0.1 and 0.2% of inorganic P ($P<0.1$), 0.3% of inorganic P or 20% of lupin ($P<0.05$). Feeding the diet with 0.3% of inorganic P resulted in leg disorders in 3 chickens,

TABLE 2

Chicken performance and phosphorus utilization indices (n = 8)

Item	Diets								Standard error of means	Significance of variance ratio (F)
	P0	P1	P2	P3[a]	LP0	LP2	BP0	BP2		
Ration characteristics										
Inorganic P addition (g/kg)	0.0	1.0	2.0	3.0	0.0	2.0	0.0	2.0		
Seeds tested[b]	–	–	–	–	L	L	B	B		
Performance										
Body weight at 21 days (g)	400	499	513	480	469	506	462	498	6.5	**
Feed efficiency, g:g	2.18	1.91	1.90	2.05	1.82	2.01	1.90	1.89	0.033	NS
Femur										
Weight (g)	1.26	1.81	1.89	1.94	1.49	1.73	1.38	1.85	0.032	**
Ash content (%)	45.8	51.8	52.5	52.0	48.1	52.5	46.3	52.8	0.39	*
(g)	0.59	0.93	0.99	1.01	0.71	0.91	0.64	0.98	0.019	**
Tibia (mean of left and right)										
Weight (g)	2.07	2.93	2.93	2.89	2.59	2.85	2.49	2.68	0.056	*
Breaking strenght (kg)	7.32	12.14	13.18	13.16	10.23	12.72	7.17	13.00	0.035	**

[a] n = 5

[b] L-lupin seeds, B-field bean seeds

** P<0.01

* P<0.05

NS P>0.05

and final body weight was lower by 6% ($P>0.05$) than of birds given the diet supplemented with 0.2% of P, which performed best.

Including 20% of lupin or field bean as protein and P sources (diets LP0 and BP0 containing P from plant sources only) gave body weight gains only 7% lower than those found in the chickens given similar diets, but supplemented with 0.2% of inorganic P (LP2 and BP2).

Feed conversion was poorer ($P>0.05$) in the chickens fed semi-synthetic diets with O and 0.3% of supplemental P. In all other groups feed conversion was very similar.

The bone mineralization indices, as femur ash deposition and tibia breaking strength improved ($P<0.01$) with increasing levels of inorganic dietary P, particularly with the first increment (diet P1). The addition of either lupin or field bean to the low P diet caused quite different responses in bone quality traits, particularly when net effects over the diet without inorganic P addition (P0) were compared. Field bean proved inferior to lupin as the source of available P, and on the field bean diet (BP0), the responses in femur ash concentration and total ash content, and tibia breaking strength over those in the chickens fed P0 diet were lower by, respectively, 78, 58 and 105% ($P<0.01$) comparing to these produced on lupin containing diet (LP0).

Total P retention ranged from 75–42% of intake and depended on dietary P level and source (Table 3). The proportion of dietary P retained was negatively correlated with the logarithm of total P intake ($r - 0.876$, $P<0.005$).

Phytic P availability ranged from 68 to 17% of intake (Table 3) and was also negatively correlated with the logarithm of total P intake ($r - 0.858$, $P<0.01$). The actual total and phytic P availability values in the chickens fed diets containing field bean (BP0 and BP2) were respectively lower by about 30 and 10% than found in the birds given diets with inorganic P, while with lupin diets the availability values were in good conformity with the levels found for inorganic P supplemented diets. Total P retention from low P diets P0, LP0 and BP0 was higher than non-phytic P intake, thus indicating some phytic P digestion and utilization. However, phytate availability was higher than 50% only when the P0 diet, deficient in both total and non-phytic P, was fed (Table 3).

Except when low P diets (P0, LP0, BP0) were fed, the total daily P retention was 0.20–0.22 g and this value probably reflects the true requirement of broilers of about 500 g body weight.

The response of body and bone weights and bone mineralization indices to total dietary P intake in the groups fed P deficient (P0) and supplemented with inorganic P (P1–P3, LP2, BP2) diets followed a logarithmic curve. The data plotted semilogarithmically produced almost straight-line responses up to 0.6% of total P (basal + 0.2% of inorganic P). The relationship between bone breaking strength and log P concentration is shown in Figure 1, and the calculated

TABLE 3

Daily total, phytic and non-phytic phosphorus balance (n = 8)

Item	Diets[b]								Standard error of means	Significance of variance ratio (F)
	P0	P1	P2	P3[a]	LP0	LP2	BP0	BP2		
Intake (g)										
total P	0.20	0.33	0.38	0.44	0.32	0.45	0.31	0.46	0.007	**
phytic P	0.10	0.12	0.12	0.12	0.18	0.19	0.17	0.18	0.002	**
non-phytic P	0.10	0.21	0.26	0.32	0.14	0.26	0.14	0.28	0.005	**
Excretion (g)										
total P	0.05	0.11	0.17	0.22	0.14	0.24	0.15	0.26	0.003	**
phytic P	0.03	0.07	0.08	0.08	0.10	0.13	0.11	0.15	0.002	**
non-phytic P	0.02	0.04	0.09	0.14	0.04	0.11	0.04	0.11	0.002	**
Difference between intake and excretion (g)										
total P	0.15	0.22	0.21	0.22	0.18	0.21	0.16	0.20	0.005	*
phytic P	0.07	0.05	0.05	0.04	0.07	0.06	0.06	0.03	0.002	**
non-phytic P	0.08	0.17	0.16	0.18	0.11	0.15	0.10	0.17	0.004	**
Availability (% of intake)										
total P	75	68	55	50	56	46	51	42	0.88	**
phytic P	68	47	37	36	44	32	32	17	0.97	**
non-phytic P	82	82	64	56	73	57	69	59	0.92	**

[a] n = 5

[b] Diet composition given in Table 1

** $P<0.01$

* $P<0.05$

availability values given in Table 4. Availability values were much higher when calculated based on body and bone weight responses than on bone mineralization indices. The sensitivity in response evaluated as a difference between the availability of P from lupin and field bean may be ordered as follows: tibia weight < body weight < femur ash deposition < femur ash concentration < tibia breaking strength. Thus, judged from breaking strength measurements the availability of P from lupin compared with inorganic P was calculated to be 53%, while for field bean it appeared slightly negative (– 2%), and no improvement in the bone breaking strength over P deficient (P0) diet was stated. When tibia weight was applied to such calculations, the respective availability values were 53 and 46% (Table 4).

In spite of differences in calculated availability values, tibia breaking strength (Y, kg) was well correlated with femur ash content (X_1, %; X_2, g), with the respective regression equations:

$$Y = 0.847\ X_1 - 31.42 \pm 0.55,\ r = 0.980$$
$$Y = 14.66\ X_2 - 1.27 \pm 0.67,\ r = 0.970$$

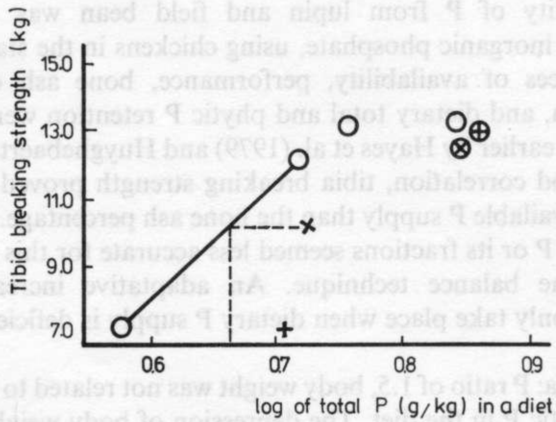

Fig. 1. The relationship between tibia breaking strength (Y) and the logarithm of total P concentration (g/kg) in the diet (X).
o – diets supplemented with ignorganic P; x – diets with lupin; + – diets with field bean
The P content of semi-syntetic diets with inorganic P, equivalent to femur breaking strength measured in the chickens fed diets with leguminous seeds (LPO and BPO) were found by a graphical method as N log of the intersept with X axis. The example illustrated is for LPO containing 5.3 g od total P (log 0.66, N log 4.6). Compare also explanation to Table 4.

TABLE 4

Phosphorus availability from lupin and field beans and from diets containing these seeds, evaluated from the response ratio, in relation to the diet containing equivalent amount of inorganic P[a]

Traits	Availability of P (%) from			
			diets with	
	lupin	field bean	lupin	field bean
Body weight 18 days	63	62	89	90
Tibia weight	53	46	87	86
Femour ash deposition	33	15	81	78
Femour ash concentration	37	10	82	77
Tibia breaking strength	53	0[b]	87	74

[a] Effective dietary P concentration (g/kg) related to the trait value was read as N log of total dietary P from the plot of the trait value (Y) vs. a log of dietary P content (X, g/kg), using a graphical method (comp. Fig. 1). The example illustrated is for LPO, the intercept of femur breaking strenght of 10.2 kg is at log 0.66, N log equal to 4.6 g of total dietary P content.
Subtracting 3.8 g (P content per 1 kg of P0 diet) from this value gives 0.8 g available out of 1.5 g of total P attributable to lupin (5.3 g per 1 kg of LP0 – 3.8 g per 1 kg of P0 diet). Availability calculations were as follows:
for lupin: $0.8 : 1.5 \times 100 = 53.3\%$
for LP0 diet $4.6 : 5.3 \times 100 = 86.8\%$.

[b] An actual small negative value (–2%) was considered zero.

DISCUSSION

The availability of P from lupin and field bean was determined by comparison with inorganic phosphate, using chickens in the starting period of growth. As indices of availability, performance, bone ash deposition and breaking strength, and dietary total and phytic P retention were used.

As was found earlier by Hayes et al. (1979) and Huyghebaert et al. (1981), in spite of their good correlation, tibia breaking strength proved more sensitive determinant of available P supply than the bone ash percentage. The calculated retention of total P or its fractions seemed less accurate for this purpose due to the errors of the balance technique. An adaptative increase in phytate digestibility can only take place when dietary P supply is deficient (Moore and Veum, 1983).

At a dietary Ca: P ratio of 1.5, body weight was not related to P supply above 0.3% of non-phytic P in the diet. The depression of body weight at the lowest dietary non-phytic P levels (diets P0, LP0, BP0) was a consequence of both Ca:P ratio and a too low P supply. Huyghebaert et al. (1981) found that at Ca:P = 1, the non-phytic P level ranging 0.14–0.35% had very little effect on body weight gain, and a diet with 0.39% of total and 0.14% of non-phytic P produced in 24-day old chickens the same body weight (515 g) as a diet with 0.3% non-phytic and 0.54% of total P and Ca:P = 1.85.

The P0 diet containing 0.19% of non-phytic P did not cause losses of chickens, though similar diets resulted in more than 60% mortality in the experiments of Hayes et al. (1979) and Osorio and Jensen (1986). Decisive in this respect could have been Ca:P ratio, much narrower in our experiment.

Irrespective of values obtained, all the traits evaluated showed much better availability of P from lupin than from field bean. Such a difference might be caused by an absence or presence of phytase activity in the feed, as for istance was found in maize and wheat (McCance and Widdowson, 1944) or in triticale (Pointillart et al., 1987). It may also arise from a different cation component in phytate salts (Waldrup et al., 1964) or be caused by the action of anti-nutritive factors, such as tannis and trypsin inhibitors. These antinutrients adversely affect digestion and enzyme action in the intestine, are present in field beans (Ernest, 1987) but are virtually absent in lupins (Hill, 1986).

Some evidence of the low digestion of phytate from field beans in 3–4 week old broilers may be derived from the results of Rubio and Brenes (1988) who found a reduction of zinc availability when raw and autoclaved field bean and their fractions were included in the diet.

The improvement of body weight in chickens given the lupin or field bean diets supplemented with 0.2% of inorganic P (diets LP2 and BP2) indicated that reduction of growth in the chickens fed LP0 and BP0 diets was caused rather by P deficiency than inclusion of legumes in the diets.

The differences in P availability from lupin and field bean assayed based on the responses of tibia ash percentage and femur breaking strength (Table 4) are difficult to explain, considering the good correlation between these traits. The determined range of bone ash percentage in the chickens given diets supplemented with 0–0.3% of inorganic P was much narrower than published for a similar P range by Osorio and Jeansen (1986) and Akpe et al. (1987) (45–54% vs. 28–50%). It seems than defatting the bones before ashing, as was done in our study and not in papers cited above, results in flattening of the slope of regression line between dietary P supply and bone ash, and thus reduces sensitivity of availability assay with this response criterion.

Our values of availability of P from leguminous seeds were lower than found by Nvokolo et al. (1976) in short-time digestibility trials with soyabean meal and other protein sources. Such discrepancies could be caused by very low Ca:P ratio in the individual feeds evaluated by these authors. At very low P intake (Ca:P = 0.2:1), phytate absorption may reach even 94% (Nahapetian and Young, 1980).

Leg lesion incidences at the highest inorganic P supplement (diet P3) are in accordance with the results of Edwards and Veltman (1983) and Halley et al. (1988). Use of NaH_2PO_4 as the P source in our study could adversely affect cation:anion ratio which seems to be involved in the mechanism of inducing of leg deformation (Halley et al., 1988). To improve the acid-base balance of the chickens, in further studies it seems advisable to use $CaHPO_4$ as the inorganic P source.

The combination in a diet of cereals and legumes showing a higher P availability, as with wheat or triticale and lupins, presumably would make it possible to obtain good body weight gains with reduced inorganic P supplementation, providing the Ca:P ratio is kept within 1–1.5.

CONCLUSIONS

1. Availability of phosphorus for starting broiler chickens was higher from lupin *(Lupinus albus)* than from field bean *(Vicia faba)*, as was shown by tibia breaking strength and femur ash content.

2. At Ca:P = 1.5, addition of inorganic P above 0.3% of dietary non-phytic level had only insignificant ($P > 0.05$) effect on the body weight of 21 day old chickens.

3. NaH_2PO_4 used as P source at the level of 0.3% of diet induced incidences of leg disorders in 37.5% of chickens.

4. Phytic P availability varied from 68 to 17%. It was negatively correlated ($r = 0.86$, $P < 0.01$) with total dietary P content.

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STRESZCZENIE

Przyswajalność fosforu z nasion bobiku *(Vicia faba)* **i łubinu** *(Lupinus albus)* **dla kurcząt brojlerów**

Oznaczono przyswajalność fosforu z nasion łubinu i bobiku w doświadczeniu przeprowadzonym na 64 kurczętach brojlerach od 0 do 18 dni życia. Jako wskaźniki zastosowano mineralizację i wytrzymałość kości na złamanie oraz retencję frakcji P.

Masa ciała kurcząt była mniejsza ($P<0,01$) przy skarmianiu półsyntetycznej i niedoborowej pod względem zawartości P dawki (P0), bez dodatku P nieorganicznego. Zawartość popiołu w kości udowej i wytrzymałość kości goleniowej na złamanie była najmniejsza i podobna u kurcząt żywionych dawkami z niedoborem P (P0) i zawierającą nasiona bobiku (BP0). Zawartość popiołu i siła łamania kości u tych kurcząt były mniejsze, odpowiednio o 12% ($P<0,05$) i 45% ($P<0,01$) niż u kurcząt żywionych dawką z 0,2% dodatkiem P nieorganicznego (P2), o najlepszej mineralizacji kości.

Fosfor był lepiej przyswajalny z nasion łubinu niż bobiku, o czym wnioskowano na podstawie wytrzymałości na złamanie – odpowiednio 53 i 0% w stosunku do P nieorganicznego przyjętego jako przyswajalny w 100%.