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The influence of the phosphorus and calcium content of feeds on growth, feed conversion and slaughter quality, and on the chemical, mechanical and histological parameters of the bone tissue of pigs

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Summary

In a 2 × 4 factorial experiment on 96 individually housed animals, two calcium/phosphorus ratios (1.3 and 1.9) and four phosphorus levels (0.4, 0.5, 0.6, 0.7 % total P) were tested. The influence of the treatments on growth, feed conversion and slaughter quality was measured. At the same time several bones of the autopodium skeleton of each animal were examined. In the radii fat free bone substance, ash, Ca, P, Mg, hydroxyproline and hexosamine were determined and bending tests were carried out on these bones. The tibias of a number of animals were examined histologically.

This experiment showed the 1.3 Ca/P ratio to be more favourable than the 1.9 ratio. A phosphorus content of 0.6 % was sufficient for good growth and favourable feed conversion. The quality of the bone tissue, for which the specific resistance to distortion and breaking was used as a standard, was close to maximum at a phosphorus level in the feed of 0.6 %. The strength of the whole bone, measured as the bending moment for distortion and breaking, was however greatest when the phospohrus level was highest, the mineralization of the bone tissue also showed a further increase when the phosphorus content in the feed was increased from 0.6 to 0.7 %.

During the experiment only a few cases of leg weakness were noted; these occurred arbitrarily throughout all the experimental treatments. On the basis of the results of this experiment, the phosphorus and calcium requirements for optimal growth and feed conversion for the modern Dutch pig are 0.6 % phosphorus and 0.78 % calcium. Higher levels of these minerals in the feed may, however, increase the deposition in the skeleton.

Introduction

The increased interest shown in recent years for an accurate estimate of the phosphorus requirement of fattening pigs is due to several factors. The increased cost of feed phosphates has led feed manufacturers to be doubly thrifty in the use of phosphorus and to investigate the feasibility of cheap sources of phosphorus. As some 80 % of the phosphorus present in the body of mammals is linked to calcium in skeleton and teeth, it is clear that in a study of phosphorus requirements calcium must also be taken into consideration. Although considerable material is available on the calcium and phosphorus requirements in pigs (Bayley et al., 1971, 1975b; Cunha, 1971; Chapman et al., 1962; Cromwell et al., 1970, 1972; Günther, 1967, 1969, 1970; Just Nielsen, 1972; Rosin, 1972; Vemmer et al., 1973), not enough is known about the phosphorus requirements of modern Dutch pure breds and cross breds. Pigs of the meat type might well have a higher calcium and phosphorus requirement (Günther et al., 1967). An insufficient supply of these minerals may lead to decreased mineralization of the bone tissue of the bones of the limbs. Distortions and curvatures of the extremities could result when the legs are subjected to straining actions. Moreover, the juvenile skeleton of these pigs carries relatively more meat, which could lead to serious locomotive disturbances. Grünhagen (1969) calls this syndrome 'leg weakness'. In a juvenile skeleton it is quite common for deviations in the form and position of forelegs and hindlegs to occur. The first symptoms can be observed in pigs weighing 35 to 40 kg. Rapid growth, in particular during the period up to 60 kg, was correlated with a low gait score (Hansen, 1974). It is probable that a complex of factors, among which breed of pig, feedstuffs, housing and management, play a role in the etiology of the syndrome 'leg weakness' (van de Kerk, 1974). The pollution of ground and surface water with phosphates, which forms such a problem at the moment (Kolenbrander, 1971) also calls for an accurate estimate of the phosphorus requirement of fattening pigs. A reduction in the phosphorus content of pig feeds could lead to a reduced amount in the manure and thus contribute to a decrease in the phosphates present in the environment. The experiment described here was undertaken not only to gain a better insight into the phosphorus requirement of the present Dutch fattening pig, but also for environmental reasons.

Material and methods

Experimental design

In a 2 × 4 factorial experiment we investigated the influence which two calcium phosphorus ratios and four phosphorus levels had on growth, feed conversion and slaughter quality. After slaughter a number of mechanical, chemical and histological parameters of the bone tissue were determined. The experiment was carried out on 96 individually housed pigs. Up to a weight of 30 kg all animals received the same starter feed. The eight experimental feeds were fed until the animals had attained a body weight of 108 kg. The trial began on 2 July 1974; the last animals reached their final weight at the beginning of October 1974. The phosphorus content in the

Table 1. Composition of the basic ration (%).

maize	30	salt	0.3
wheat	10	chalk	1.0
barley	10		
tapiocameal	15	calculated contents:	
wheat middlings	10	crude protein	15.4
soybeanmeal	17.5	lysin	0.75
alfalfameal	2.5	methionin + cystin	0.53
molasses	2.5	Ca	0.52
animal fat	0.7	P	0.40
trace elements-vitaminpremix	0.5	unit of energy value for pigs	1.04

experimental feeds was increased in four equal stages from 0.4 % to 0.7 %, for the calculated Ca/P ratio of 1.3 as well as for the Ca/P ratio of 1.9. The feeds containing 0.4 % phosphorus had no inorganic phosphate added. The feeds with a higher calcium and/or phosphorus content were obtained by replacing the maize in the basal ration (calculated phosphorus 0.4 %; calculated calcium 0.52 %) by calcium carbonate and/or dicalcium phosphate. The composition and the calculated contents of the basic ration are given in Table 1. The chemical analyses of the eight experimental feeds used can be found in Table 2. The experiment used two purebred pig breeds common in the Netherlands: the Dutch Land Race (NL) and the Great Yorkshire (GY), and one cross bred obtained from these two breeds (GY × NL). Four litters of each type were used and eight animals from each litter, so that all eight trial feeds could be tested on each litter.

The stock was choosen in such a way as to obtain a cross section of the pure breds and cross breds most common in the Netherlands. The experiment was not intended to determine whether pigs of the two breeds and the cross bred reacted differently to the experimental treatment.

At a live weight of 55 kg one litter of eight animals from each of the two pure breds and the cross bred were slaughtered. This was intended to ascertain whether there were indications that at this weight disturbances in the mineralization and

Table 2. Chemical analyses of the 8 experimental feeds (%).

Calculated								
Ca/P ratio	1.3	1.3	1.3	1.3	1.9	1.9	1.9	1.9
phosphorus level	0.4	0.5	0.6	0.7	0.4	0.5	0.6	0.7
Analysed contents								
Ca	0.53	0.62	0.75	0.86	0.72	0.88	1.06	1.26
P	0.43	0.50	0.61	0.70	0.40	0.50	0.60	0.70
Ca/P ratio	1.23	1.24	1.23	1.23	1.80	1.76	1.77	1.80
mositure	12.4	12.4	12.2	12.2	12.2	12.4	12.2	11.8
crude protein	15.0	14.4	14.8	14.8	14.5	14.7	14.6	14.2
crude fibre	4.4	4.5	4.5	4.6	4.6	4.2	4.4	4.4

histological and mechanical characteristics of bone tissue occurred as a result of the experimental treatment. The remaining 72 animals were slaughtered when they had reached a weight of approximately 108 kg.

The animals were fed according to body weight. At 30 kg the pigs on the basal ration received 1.4 kg feed. The feed quantity was increased as body weight rose, and at 100 kg the animals received 3.2 kg. In the case of the remaining test groups the feed quantity was corrected for replacing the maize by calcium carbonate and/or dicalcium phosphate, so that all the pigs received isocaloric feed quantities. This feeding scheme is 90 to 95 % of ad libitum feeding. Any possible differences between the various feeds will be more likely to be reflected on this scheme than when the animals are kept on a more restricted feeding scheme. Feeds were administered as pellets. During the experiment the pigs were weighed once every two weeks.

The mechanical examination of bones and bone tissue

In a living pig, four kinds of straining action exercise and influence on the bones of the limbs: axial compression, axial tension, bending and twisting moments. In a long bone the cartilage tissue of the joint surfaces as well as the epiphyseal plates form a locus minoris resistentiae. The bone tissue is also fairly inhomogeneous material. The force which is necessary to break or permanently deform a given bone will then depend on the point where pressure is exerted on the bone. Of all these forces and moments only a bending moment was measured. The bending tests were, in spite of the limitations, carried out with radii from the pigs slaughtered at 55 kg as well as from those slaughtered at 108 kg. One of the radii was used for the bending test and the other for chemical analysis. After these bones had been divested of muscle and connective tissue, they were immediately tested, so as to prevent any deviations in the results due to desiccation. Before the experiment the radii were divided into proximal and distal epiphyseal ends and middle section (metaphyse and diaphyse together). The middle sections taken from the pigs slaughtered at 55 kg were embedded in synthetic resin at the ends (that is, at the epiphyseal plates). This was done in order to prevent the specimen from rotating when pressure was applied. In addition, by fastening the ends in this way it was possible to maintain an accurately measurable distance (l in Fig. 1) (Weir et al., 1949; Miller et al., 1962). In the case of the animals slaughtered at a live weight of 108 kg this procedure was omitted, as there were indications that it would have an unfavourable effect on the measurements. The bending tests carried out on the bones of all 96 animals were done by the Metaal-Instituut TNO (Metal Research Institute TNO) in Apeldoorn, with the aid of an Amsler tensile test machine equipped with a dial gage. This equipment measured the force exerted on the bone section, which was placed on rollers, as a function of the displacement. The principle of the measurement is given in Fig. 1.

A load-strain diagram was made of one radius from each animal (Fig. 2) by which the distortion strain (F_a, that is, the point at which permanent distortion occurs) and the maximum strain (F_{max}, that is, the load at which the bone breaks) were determined. The distortion strain F_a was determined because it was thought that this factor might be connected with what happens in the live animal when distortions and curvatures of the extremities occur. The pressures F_a and F_{max} ob-

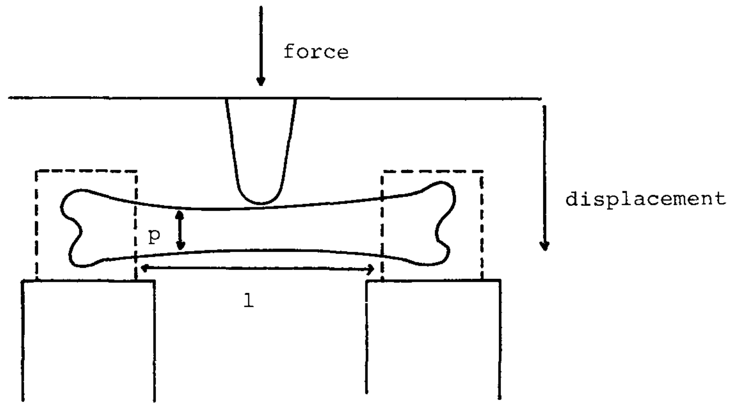

Fig. 1. Schematic representation of the principle of threepoints bending tests.
l = distance between supporting divices; p = smallest ouside diameter.

tained from the bending tests are certainly not conclusive, as they are dependent on the shape of the bone section as well as the distance between supporting devices. This distance was set before each bending test.

In order to determine the shape of the bone section at the point of fracture, the

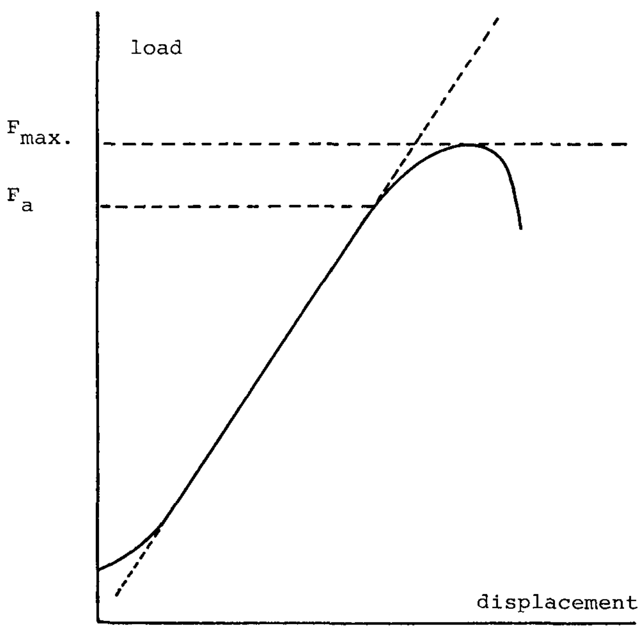

Fig. 2. Load-strain diagram.

broken ends were matched together and then completely embedded in synthetic resin. A disc was then sawed out of the middle part of the bone section embedded in synthetic resin. This disc was photographed and a linear enlargement V was made in order to obtain the necessary accuracy of measurement. With the aid of a planimeter the total surface of the bone cross section (U_1) and the surface of the marrow section (U_2) were measured on the enlargement. The surface of the cortex cross section (mm^2) was calculated from the formula $(U_1 - U_2)/V^2$.

Using the loads F_a and F_{max} and the surface of the cortex cross section, the bending moment M_a (bending moment at which permanent distortion occurred) and M_{max} (bending moment at breaking) were calculated in Nm, as well as the specific resistances R_a (to distortion) and R_{max} (to breaking) in N/mm^2. The bending moment can be seen as a measure of the strength of the bone as a whole, and the specific resistance is a measure of the strength of the bone tissue. The abovementioned quantities were calculated in the following ways. The bending moments M_a and M_{max} in Nm: $Ma = (F_a \times l)/4$ and $M_{max} = (F_{max} \times l)/4$, in which l is the distance between supporting devices (Fig. 1).

For the specific resistance we first had to calculate the moment of inertia and the moment of resistance of the bone section. The moment of inertia I (mm^4) was calculated as $I = \pi (D^4 - d^4)/64$. Here D and d are, respectively, the outside diameter of the broken bone section and the diameter of the marrow portion of the same bone section. We have assumed that the bone section had roughly the form of a cylindrical pipe. D and d were determined in two different ways, namely from $D = 2 (U_1/\pi)^{1/2}$ and $d = 2 (U_2/\pi)^{1/2}$, and by direct measurement from the average of the diameters on the photograph in four different directions. The average of the two values for D and d thus obtained per diameter was used to calculate I.

The resistance moment (W) to bending in mm^3 was obtained from the formula $W = I/(0.5\ P)$. P is here the smallest outside diameter measured in the middle of the radius, as indicated in Fig. 1. This smallest outside diameter was selected because the bone sections used in the bending tests were placed on the slightly flat median side.

Finally, the specific resistances to permanent distortion (R_a) and breaking (R_{max}) in N/mm^2 were calculated as follows: $R_a = 1000\ M_a/W$ and $R_{max} = 1000\ M_{max}/W$.

Chemical analysis

The bones of the autopodium skeleton are an important depot for the elements calcium, phosphorus and magnesium. They are the first bones to react to changes in the quantity of calcium and phosphorus in feeds (Günther et al., 1967b). For this reason the chemical analysis made use of the diaphyse and metaphyse of one of the radii, divested of muscle and connective tissue. These bone sections were weighed in water and in air to determine the volume. Then the bone sections were placed in a freeze drier and dried at —20°C for 24 hours. They were then extracted with diethyl ether in a Soxhlet for 8 hours, after which they were dried and weighed to determine the total fat-free bone substance. After grinding we then determined: the ash by weighing after heating at 550 °C for three hours; calcium and magnesium in a hydrochloric acid solution of the ash by means of atom absorption spectrophotometry; phosphorus in the hydrochloric acid solution of the ash by means of colorimetry (phosphorus vanadomolybdate complex); crude protein according to Kjeldahl; hydroxyproline and hexosamine after hydrolysis of the tissue for 15 hours with 4 *N* HCl; according to Stegeman & Stalder (1967) and Boas (1953), respectively. The hydroxyproline content can be seen as a measure of the collagen fibres and the hexosamine content as a measure of the quantity of proteoglycans (an intermediate substance between the collagen fibres).

Morphological data

Because the juvenile pig skeleton can be permanently overtaxed by rapid growth, we attempted to ascertain whether the different feeds administered at an early age produced deviations of the joint cartilage, the epiphyseal plate and the cortex of the diaphyse (Dämmrich et al., 1972). For this purpose 24 pigs were slaughtered at 55 kg and the tibiae of these animals were analysed histologically at the Veterinary Pathological Institute of the Freie Universität Berlin (Free University of Berlin) by Prof. K. Dämmrich. The techniques used are described by Dämmrich et al. (1972) and by Rodenhoff & Dämmrich (1973). To get some idea of the growth in length various parameters were analysed. First the length of the tibiae was measured. On the proximal epiphyseal plate the length of the longitudinal cartilage cell columns and the thickness of the primary and secondary spongiosa were measured in μm. At the same time X-rays were used to determine the density of the trabeculae in the primary and secondary spongiosa. A subjective norm was used here: + + signified the presence of a great many trabeculae, and + meant that only a few trabeculae had been formed. We measured the thickness of the joint cartilage, the epiphyseal cartilage and the supporting spongiosa. We also studied the growth by apposition of the tibiae. For this purpose a 90 μm thick bone disc was cut from the diaphyse and the changes demonstrated by microradiographic studies. The appositional growth of these bones was determined by the parameters: surface of the diaphyse, surface of the cortex expressed as a percentage of the total surface of the diaphyse, number of tangential lamellae and width of these lamellae.

Evaluation of the slaughter quality

Only those animals slaughtered at 108 kg were subjected to this evaluation. The day after slaughter carcass length and back fat thickness were measured. Then the animals were dissected and the sections divided into meaty parts, fatty parts and remaining parts. Then the meat percentage, fat percentage and percentage of remaining parts were calculated. The killing-out percentage was calculated on the basis of the cold slaughter weight and the last live weighing during the experiment.

Statistical analysis

The statistical analyses of the 24 animals slaughtered at 55 kg and the 72 animals slaughtered at 108 kg were carried out separately. These analyses were intended to discover whether there were differences between the two Ca/P ratios and between the four phosphorus levels. We determined whether a possible phosphorus effect could be described by a linear, a quadratic or a cubic relation, and whether there were interactions between the phosphorus effects thus described and the Ca/P ratios. By means of covariance analysis a correction was made for unequal distribution of sows and barrows throughout the treatments and for the possible influence of the live weight on the characteristic at the time the characteristic was measured. The testing of the effects was carried out with a T-test.

Results of the experiment

As the interactions between the Ca/P ratio and the phosphorus levels were generally slight and seldom significant, we considered it sufficient to cite in the tables only the main effects. The nature of the interaction is described in the text when warranted by the statistical analysis.

Growth, feed conversion and slaughter quality

The influence of the experimental treatment on growth and feed conversion in the 72 animals slaughtered at 108 kg is given in Table 3. This shows that in the period from 0 to 4 weeks the Ca/P ratio had a definite influence on growth and feed conversion. The lowest ratio produced distinctly better growth and feed conversion during this period. The same tendency was evident in the later 4-week periods. During the whole fattening period, from 30 to 108 kg, the feed conversion on the

Table 3. Influence of the experimental treatments on growth (g/day) and feed conversion in the 72 pigs slaughtered at 108 kg.

	Ca/P ratio		Significance of difference	Phosphorus level (%)				Significance	
	1.3	1.9		0.4	0.5	0.6	0.7	linear effect	quadratic effect
0-4 weeks									
growth	613	574	**[1]	587	588	609	590		
feed conversion	2.52	2.63	**	2.65	2.60	2.48	2.57	*	*
4-8 weeks									
growth	743	736		714	759	744	741		
feed conversion	2.87	2.89		2.98	2.82	2.85	2.89		*
8-12 weeks									
growth	880	880		836	894	888	901		
feed conversion	3.01	3.07		3.19	2.99	2.99	2.99	*	
12 weeks- end									
growth	886	873		856	890	880	892		
feed conversion	3.22	3.29		3.34	3.21	3.24	3.24		
whole fattening period									
growth	776	759		749	776	773	772		
feed conversion	2.98	3.05	*	3.09	2.98	2.99	3.01		*

[1]** and * significant at less than 1 and 5 % levels of probability, respectively. No indication: not significant at 5 % level of probability.

Table 4. Influence of the Ca/P ratio and the phosphorus level on the slaughter results of the 72 animals slaughtered at 108 kg.

	Ca/P ratio		Significance of difference	Phosphorus level %				Significance	
	1.3	1.9		0.4	0.5	0.6	0.7	linear effect	quadratic effect
carcass length (cm)	84.1	84.1		84.2	83.9	84.8	83.6		
back fat thickness (cm)	2.97	3.05		3.02	3.03	2.94	3.02		
killing out percentage	76.8	77.0		76.7	77.3	77.3	76.4		*
meat percentage	62.0	62.0		61.8	62.0	62.2	62.0		
fat pork percentage	29.8	29.9		30.1	29.9	29.7	29.7		
percentage of remaining parts	8.2	8.1		8.0	8.2	8.2	8.2		

Table 5. Influence of the Ca/P ratio and the phosphorus level on several physical and mechanical characteristics of the radii of the 24 pigs slaughtered at 55 kg.

	Ca/P ratio		Significance of difference	Phosphorus level (%)				Significance	
	1.3	1.9		0.4	0.5	0.6	0.7	linear effect	quadratic effect
surface of the diaphyse cross section (mm^2)	207	209		208	200	207	217		
surface of the cortex (mm^2)	116	117		125	106	113	123		*
surface of the cortex expressed as a percentage of the surface of the diaphyse cross section	56.4	56.1		61.0	53.4	54.6	56.7		
volume (ml)	26.7	26.8		27.0	25.9	26.8	27.4		
bending moment to distortion, M_a (nm)	38.48	33.85		36.30	36.77	33.15	38.08		
bending moment to breaking, M_{max} (nm)	44.79	43.73		40.91	43.25	44.41	48.84		
specific resistance to distortion, R_a (n/mm^2)	77.2	69.7		73.2	80.0	69.4	70.4		
specific resistance to breaking, R_{max} (n/mm^2)	89.5	90.0		81.2	94.0	93.3	89.1		

lower Ca/P ratio was significantly better than on the higher ratio. Growth was also better on the lower Ca/P ratio but the difference was not significant.

At the beginning of the fattening period (from 0 to 4 weeks), the most favorable feed conversion and growth rate were obtained on 0.6 % phosphorus. Later in the fattening period 0.5 % phosphorus in the feed produced a minimum for feed conversion. The 24 animals slaughtered at 55 kg showed exactly the same results for growth and feed conversion as the not slaughtered animals during the first 4 weeks of the feed trial. The slaughter results of the 72 animals slaughtered at 108 kg are given in Table 4. Only the killing-out percentage was better in the case of pigs fed on 0.5 and 0.6 % phosphorus than in the case of those receiving rations with 0.4 and 0.7 % phosphorus. Neither the Ca/P ratio nor the phosphorus level in the feed had any influence on the other parameters.

Examination of the bones

Several physical and mechanical characteristics of the radii were measured and calculated both for the 24 animals slaughtered at 55 kg and those slaughtered at 108 kg. The average results of the various treatments are given in Table 5 (24 animals weighing 55 kg) and Table 6 (72 animals weighing 108 kg).

The measurements of the bones of animals slaughtered at an early age (Table 5) produced few significant data. Only the surface of the cortex proved to be minimal when 0.5 % phosphorus was fed. The differences between the various experimental treatments were more pronounced in the case of the 72 animals slaughtered at 108 kg. The surface of the cortex, and also this surface expressed as a percentage of the diaphyse cross section, were significantly larger when the Ca/P ratio was 1.9 than when it was 1.3. The bending moments to distortion as well as to breaking were approximately the same for both ratios, with a slight tendency towards a higher value for the lowest Ca/P ratio.

For this reason the specific resistances to distortion and breaking were higher for the Ca/P ratio of 1.3 than for that of 1.9. In the case of animals slaughtered at 108 kg, the surface of the cortex of the bone section increased noticeably as the phosphorus level increased. In the surface of the cortex, which had already shown a difference between the two Ca/P ratios, there was a definite interaction between phosphorus level and Ca/P ratio. In the case of the 1.3 Ca/P ratio the maximum surface area was attained quite rapidly (between 0.5 and 0.6 % phosphorus). In the case of the 1.9 Ca/P ratio the rise continued up to the highest phosphorus level, while the maximum difference between the highest and lowest phosphorus levels was significantly greater for the highest Ca/P ratio. The bending moments at breaking and distortion also increased significantly as the phosphorus content increased from 0.4 to 0.7 % total phosphorus, although for the bending moment for breaking the increase registered between 0.6 and 0.7 % phosphorus was significantly smaller than between, for instance, 0.5 and 0.6 % phosphorus. The specific resistance to breaking and distortion – the strength of the bone tissue – also increased noticeably at the lower phosphorus levels. At 0.6 % phosphorus in the feed the maximum had just about been attained, 79 and 107 N/mm^2 for specific resistance to distortion and specific resistance to breaking, respectively. The data for the 24 animals slaughtered

Table 6. Influence of the Ca/P ratio and the phosphorus level on several physical and mechanical characteristics of the radii of the 72 pigs slaughtered at 108 kg.

	Ca/P ratio		Significance of difference	Phosphorus level (%)				Significance	
	1.3	1.9		0.4	0.5	0.6	0.7	linear effect	quadratic effect
surface of the diaphyse cross section (mm^2)	256	258		244	255	261	268		
surface of the cortex (mm^2)	161	170	*	156	162	167	175	* *	
surface of the cortex expressed as a percentage of the surface of the diaphyse cross section	63.1	65.6	*	64.0	63.8	64.2	65.4		
length (mm)	99	98		99	99	98	99		
weight (g)	58.8	58.7		55.1	58.8	59.6	61.8	* *	
volume (ml)	43.2	43.0		42.6	43.4	42.7	43.8		
bending moment to distortion, M_a (nm)	45.27	44.44		38.35	44.20	47.75	49.16	* *	
bending moment to breaking, M_{max} (nm)	60.42	59.32		48.22	59.01	64.68	67.57	* *	*
specific resistance to distortion, R_a (n/mm^2)	77.5	73.1		69.5	75.9	79.0	76.7	*	
specific resistance to breaking, R_{max} (n/mm^2)	103.3	97.2	*	87.7	100.9	107.0	105.4	* *	*

Table 7. Effect of the treatments on the chemical composition of the metaphyse and diaphyse of the radii of the 24 animals slaughtered at 55 kg.

	Ca/P ratio		Significance of difference	Phosphorus level (%)				Significance	
	1.3	1.9		0.4	0.5	0.6	0.7	linear effect	quadratic effect
defatted bone substance (g)	16.8	16.9		15.5	16.3	17.4	17.8	*	
ash[1]	65.6	65.1		64.1	65.5	65.6	65.9	*	
Ca[1]	23.5	23.3		23.0	23.5	23.5	23.5	*	
P[1]	11.6	11.5		11.2	11.6	11.6	11.7	*	
Mg[1]	0.44	0.41	* *	0.384	0.422	0.437	0.442	* *	*
hydroxyproline[1]	3.12	3.17		3.22	3.16	3.10	3.12		
hexosamine[1]	0.234	0.225		0.216	0.225	0.239	0.234		
hydroxyproline/hexosamine	13.5	14.2		15.0	14.1	13.2	13.4		
quantity of ash (g)	11.0	11.0		9.9	10.7	11.4	11.8	*	
quantity of Ca (g)	3.94	3.93		3.56	3.84	4.09	4.20	*	
quantity of P (g)	1.95	1.94		1.74	1.89	2.03	2.09	* *	
quantity of Mg (mg)	74	69	*	59	69	76	79	* *	
Ca/P ratio	2.02	2.03		2.05	2.03	2.02	2.01	*	

[1] % in defatted bone substance.

at 55 kg are roughly the same as for those slaughtered at 108 kg as regards the specific resistance to breaking and the bending moments at breaking. The data resulting from the statistical analysis were less pronounced, which is undoubtedly due to the smaller number of animals slaughtered at 55 kg (only 24) and the relatively greater variation which these animals showed in the measurement results. It is probable that the process of embedding in synthetic resin also had an unfavorable effect especially on the bending moment for distortion and the specific resistance derived in part from it.

The chemical composition of the bones of the autopodium skeleton was very clearly influenced by the phosphorus level, in the young animals (Table 7) as well as the older animals (Table 8).

The bones of the limbs appeared to grow more rapidly, and to be better mineralized when there was hy higher level of calcium and phosphorus in the feed, for not only the total quantity of bone tissue per bone, but also the level of mineral components calcium, phosphorus and magnesium clearly rose as the level of phosphorus and calcium in the feed rose. Only the magnesium content and the total quantity of magnesium present in the bone were significantly influenced by the Ca/P ratio in the feed. These two characteristics were higher for the 1.3 Ca/P ratio than for the 1.9 ratio. The Ca/P ratio in the bone itself proved to be fairly constant. The phosphorus level also influenced the parameters for the bone matrix. As the phosphorus level rose the percentage of hydroxyproline in the fat-free bone substance decreased, and the percentage of hexosamine increased. The ratio hydroxyproline/hexosamine decreased. The hydroxyproline content reacted slightly differently to the two Ca/P ratios. The 1.3 Ca/P ratio caused the hydroxyproline content to decline only slightly after 0.5 % phosphorus, while the 1.9 Ca/P ratio caused the decline to continue linearly through all levels from 0.4 to 0.7 % phosphorus.

The results of the histological study of the tibiae of the 24 animals slaughtered at 55 kg are given in Table 9. As the formation of bone tissue takes place in two different ways, the observations were carried out with respect to both periosteal and endochondral ossification and epiphyseal ossification. The bone formation, which is responsible for growth in the length of the bone, takes place primarily in the epiphyseal plates and only to a slight extent through transformation of joint cartilage into bone tissue. As regards the characteristics of this bone formation, it appears that the experimental treatments had no effect on the length of the tibia, nor on the length of the radius, measured during the mechanical examination of the animals slaughtered at 108 kg (Table 6). Of the other parameters for the tibia, only the lengt of the secondary spongiosa increased linearly as the phosphorus level rose. The density of the trabeculae formed in this layer also increased. At 0.7 % phosphorus in the feed there were far more trabeculae present (more animals with the subjective assessment + +) than at 0.4 % phosphorus (when there were more animals with +).

The length of the cartilage cell columns was minimal at 0.6 % phosphorus. These cartilage cell columns showed no irregularities and ran parallel to the longitudinal axis of the bone. During the study of the transformation of joint cartilage into bone

Table 8. Effect of the treatments of the chemical composition on the metaphyse and diaphyse of the radii of the 72 pigs slaughtered at 108 kg.

	Ca/P ratio		Significance of difference	Phosphorus level (%)				Significance	
	1.3	1.9		0.4	0.5	0.6	0.7	linear effect	quadratic effect
defatted bone substance (g)	30.9	30.6		26.5	30.9	31.9	33.7	* *	*
ash[1]	66.39	66.41		65.10	66.61	66.80	67.10	* *	*
Ca[1]	24.31	24.37		24.06	24.39	24.42	24.48	* *	
P[1]	11.52	11.47		11.20	11.52	11.64	11.62	*	
Mg[1]	0.466	0.446	*	0.416	0.453	0.475	0.481	* *	
crude protein[1]	31.72	31.73		32.82	31.59	31.47	31.02	* *	
hydroxyproline[1]	3.37	3.38		3.50	3.38	3.33	3.29	* *	
hexosamine[1]	0.209	0.207		0.197	0.201	0.217	0.218	* *	
hydroxyproline/hexosamine	16.3	16.7		18.1	17.1	15.5	15.2	* *	
quantity of ash (g)	20.53	20.37		17.27	20.60	21.29	22.64	* *	*
quantity of Ca (g)	7.51	7.47		6.38	7.55	7.78	8.26	* *	*
quantity of P (g)	3.57	3.52		2.98	3.57	3.71	3.92	* *	*
quantity of Mg (mg)	145	138		111	140	151	162	* *	*
quantity of crude protein (g)	9.78	9.69		8.70	9.77	10.01	10.45	* *	
Ca/P ratio	2.11	2.13		2.15	2.12	2.10	2.11		

[1] % in defatted bone substance.

Tabel 9. Influence of the Ca/P ratio and the phosphorus level on the results of the histologycal study of the tibiae of the 24 pigs slaughtered at 55 kg.

	Ca/P ratio		Significance of difference	Phosphorus level (%)				Significance	
	1.3	1.9		0.4	0.5	0.6	0.7	linear effect	quadratic effect
growth in length									
length of tibia (mm)	156	158		157	156	155	160		
Proximal epiphyseal plate									
length of cell (μm)	814	810		825	790	764	869		
thickness of primary spongiosa (μm)	798	761		847	768	731	772		*
thickness of secundairy spongiosa (μm)	5610	4643		4730	4735	4876	6365	*	
proximal joint cartilage									
thickness of joint cartilage (μm)	316	339		309	315	362	324		
thickness of epiphyseal cartilage (μm)	440	462		449	455	512	388		
length of the supporting spongiosa (μm)	1478	1272		1454	1408	1540	1098		
growth by apposition									
surface of diaphyse (mm^2)	234	239		242	234	232	237		
surface of cortex (mm^2)	165	177		173	167	171	173		
surface of the cortex expressed as a percentage of the surface of the diaphyse	70.9	74.4		71.6	71.7	74.0	73.4		
ber of tangential lamellae (n)	21.2	20.2		20.1	20.6	21.2	20.8		
width of the tangential lamellae (μm)	118	120		121	118	122	117		

tissue, no influence of the experimental treatments could be discovered on the following parameters: thickness of the joint cartilage, thickness of the epiphyseal cartilage and length of the supporting spongiosa. Neither did the growth by apposition appear to be influenced by the experimental treatments. The tangential lamellae of all the tibiae examined consisted of a mineralized nucleus which was covered with lamellar bone tissue with a lower mineral content. Disturbances in mineralization processes could not be established histologically.

During the experiment symptoms of leg weakness were discovered in about ten animals. One animal showed quite serious symptoms. However, no relation could be established between these abnormalities and the mineral content and/or the Ca/P ratios of the trial feeds.

Discussion

In determining the phosphorus requirement, one can make use of various methods and criteria. It is possible to investigate how growth and feed conversion react to increasing quantities of phosphorus in the feed and in this way determine the optimal quantity of phosphorus. Even then the rations can be composed in various ways: it is possible to vary the phosphorus level while calcium remains at one or more constant levels (Bayley et al., 1971; Cromwell et al., 1970; Miller et al., 1964; Bayley & Thomson, 1969; Bayley et al., 1975a). But it is also possible, as was done during the study under discussion, to choose one or more constant Ca/P rations for all phosphorus levels (Vemmer et al., 1973; Mudd et al., 1969; Schroeder et al., 1974; Bunch et al., 1969a, 1969b). Besides the criteria growth and feed conversion, almost all workers also carried out measurements on parts of the skeleton, such as strength measurements and mineralization. Some workers measured calcium and phosphorus retention (Vippermann et al., 1971; Mudd et al., 1969; Just Nielsen, 1972). A fairly accurate idea of the mineral retention can also be obtained by measuring not only the ash content of the bones but also the total quantity of minerals in a given bone.

The above data indicate that non-synthetic rations should contain 0.6 % phosphorus at the beginning of the fattening period and 0.4 to 0.5 % later on, in order to assure a favourable feed conversion. On the basis of retention trials, however, Just Nielsen (1972) recommends somewhat higher percentages, especially for maize/soybean rations. Furthermore, an excessive calcium level has been proved to have an unfavourable effect, especially when the phosphorus level was low (Cromwell et al., 1970). A Ca/P ratio of 1.3 would seem generally favourable (Chapman et al., 1962). From the results of the experiment under discussion it can be concluded that in order to obtain good growth and a favourable feed conversion in the modern Dutch fattening pig, feed should contain at least 0.6 % phosphorus at the beginning of the fattening period and 0.5 % during the final part. A phosphorus level of 0.7 % was shown to have an unfavourable effect on feed conversion, growth and killing-out percentage. Generally speaking, more calcium and phosphorus in the feed are necessary for optimal mineralization of the bone tissue of the autopodium skeleton, than for optimal feed conversion and growth.

The bending moments for breaking and distortion, which indicate the strength of the total bone section, are greatest at 0.7 % phosphorus and show an increase along all phosphorus levels investigated from 0.4 to 0.7 % phosphorus. The latter is contrary to the results of an experiment carried out by Bayley et al. (1975b), in which only the boars showed an increase in the force needed to break the femur, when phosphorus content in the feed was raised from 0.4 % to 0.6 % and the calcium level remained constant. This increase had no effect on sows, while further increase up to 0.8 and 1.0 % phosphorus did not produce stronger femurs either in boars or sows.

The experiment under discussion shows that the quality of the bone tissue expressed as specific resistance to distortion and breaking reaches maximum when the phosphorus level in the feed is just over 0.6 %. The greater bending moment obtained by further increasing the phosphorus and calcium levels is the result of a greater cortex surface. This enlarged cortex surface did not lead to a proportionate increase in the strength of the bone section.

As the phosphorus content in the feeds increased, the bones of the autopodium skeleton showed a better mineralization (Table 7 and 8). At a 0.7 % phosphorus level the percentage of ash even attained 67.1 % at the end of the fattening period. Currey (1967a) considers this an optimal ash content. According to Vose & Kubala (1959) this is accompanied by an increased breaking strength. In the present experiment, too, the strength of the total bone section was greatest at a phosphorus level in the feed of 0.7 %. No attempt was made to ascertain whether strength continued to increase as the phosphorus levels in the feed rose. According to Mudd et al. (1969); Vemmer et al. (1973) and Just Nielsen (1972), this would seem to be the case.

With respect to the Ca/P ratio in the feed, it is clear that a surplus of calcium has an unfavourable effect on feed conversion as well as on the specific resistance of the radius to breaking. In general a surplus of calcium has a negative effect on phosphorus utilization. Bayley et al. (1975a) carried out similar observations, concentrating in particular on young, fast-growing pigs. The Ca/P ratio in the bone tissue was fairly constant, which is in agreement with the observations of Weniger & Funk (1953) & Oslage (1964). There did appear to be a tendency for this ratio to be lower as the phosphorus level increased and as the Ca/P ratio in the feed was lower. This would give an explanation for the increased magnesium level in the bone tissue. According to Neumann & Neumann (1958) an increase in the phosphorus content of the feed produces more phosphate in the apatite crystals. This is accompanied by an increase in the number of negative valencies on the surface of these crystals, by which more magnesium ions from the extracellular liquid can be bound.

Chemical examination of the bone tissue also showed that as the phosphorus level in the feed increased the ratio hydroxyproline/hexosamine altered. The higher the phosphorus level, the narrower the ratio. In the matrix of the bone the amorphous basic substance appears to increase as the phosphorus level rises. This increase in mucopolysaccharides coud mean that mineralization of bone tissue can increase, as these proteoglycans play an important role in this process. The acid mucopolysaccharides fix the calcium ions with the aid of sulphate groups from the chondroitin sulphuric acid, while the phosphate is bound to the neutral mucopoly saccharides. The chemical analyses carried out during the present experiment (Table 7 and 8) indicate that mineralization of the bone tissue increases as the phosphorus level in the feeds rises. This is supported by the histological observations.

A higher phosphorus content in the feed produced, at any rate in the animals slaughtered at 108 kg, a larger cortex surface, resulting in increased breaking strength, which was obvious from the variations in the bending moments (Table 6). This was also established by Schroeder et al. (1974). But it is questionable whether it is necessary to strive for maximum deposit of phosphorus and calcium in the skeleton. It would seem preferable to choose a level which allows for maximum strength of the bone tissue. In the present experiment this level was almost exactly 0.6 % phoshorus. Not only for maximum strength of the bone tissue is 0.6 % phosphorus necessary, but also for a maximum growth with a minimum feed conversion, especially for the young pig below the live weight of 50 kg. Another reason for the choice of this phosphorus level is the variation in availability of the plant phospohrus. A basis of inorganic phosphorus wille be more safe for the practical pig feeding.

This manner of fixing standards has, however, one disadvantage. It is possible that the leg weakness syndrome is suppressed somewhat by administering excessive quantities of calcium and phosphorus. It is true that according to Vaughan (1971) the calcium and phosphorus levels have no influence on this abnormality, but we are not acquantied with any extensive research on the subject. According to the measures used in the experiment, it is at least possible to strengthen the bones by increasing the phosporus and calcium in the feed.

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