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Effects of *Aspergillus fumigatus* phytase on phosphorus digestibility, phosphorus excretion, bone strength and performance in pigs

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Abstract – Phytic-phosphorus has a very low bioavailability for monogastric animals and the non-utilized mineral contributes to the phosphorus (P) pollution problems. Phytases may ameliorate phytic-P antinutritive properties. However, phytases are very sensitive to the pelleting temperature commonly used for compound feed production and thus the challenge to produce a more thermostable phytase is very important. Pure *Aspergillus fumigatus* phytase (AFP) has the ability to refold into a native-like fully active structure after heat denaturation (20 min at 90 °C). The aim of the present work was to evaluate in vitro (in feed) and in vivo in young and in growing-finishing pigs the effects of AFP included in the feed at a level of 500 U/kg. Feed supplementation with AFP resulted in an in vitro phosphorus release of about three times higher than that obtained from the basal diets, irrespective of the pH value used for the determination (5.5 or 7). When the supplemented feed was steam pelleted at about 84 °C, the free P obtained after incubation at pH 5.5 represented 53 % on an average of that obtained from the corresponding mash diets. The phytic-P-rich diets systematically induced hypophosphataemia, hypercalcaemia and hyperphosphatasaemia. The normal blood levels of P, Ca and alkaline phosphatase were restored by AFP. P apparent digestibility was significantly higher for the AFP diet (52.8 versus 30.8 %). The improvement in Ca digestibility was not statistically significant. In all three in vivo experiments, AFP significantly decreased the P concentration in faeces (between 13 and 33 %) as well as increased the growth rate and decreased the feed conversion ratio. Bone strength was significantly higher in the growing-fattening pigs fed on the AFP diet. © Inra/Elsevier, Paris

phytase / *Aspergillus fumigatus* / phosphorus / digestibility / swine / performance

Résumé – Effets de la phytase d'*Aspergillus fumigatus* sur la digestibilité et l'excrétion du phosphore, la résistance osseuse et les performances chez le porc. Le phosphore (P) phytique a une très faible biodisponibilité pour les animaux monogastriques et le P non utilisé contribue aux problèmes de pollution. Les phytases peuvent réduire ces inconvénients. Toutefois, ces enzymes sont très sensibles à la granulation à chaud des aliments composés. Ainsi, l'obtention d'une phytase résistante à

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haute température serait très utile. La phytase d'*Aspergillus fumigatus* (AFP) a la capacité de se replier en une protéine active et similaire à la naturelle après dénaturation à chaud (20 min à 90 °C). Les effets d'AFP (500 U/kg d'aliment) ont été évalués in vitro et in vivo chez le porcelet et chez le porc en croissance. L'addition d'AFP aux régimes alimentaires de base a produit une libération de P in vitro approximativement trois fois supérieure à celle obtenue à partir des aliments de base, indépendamment du pH final d'incubation (5,5 or 7). Après granulation à chaud l'activité de l'enzyme n'a été fortement réduite (environ 47 %) que pour une température de 84 °C. Les régimes de base, riches en P-phytique ont systématiquement induit une hypophosphatémie, une hypercalcémie et une hyperphosphatasémie. Les valeurs physiologiques de ces paramètres ont été restaurées par AFP. La digestibilité fécale apparente du P a été très significativement améliorée (52,8 % versus 30,8 %) par AFP. AFP a réduit très significativement la concentration de P dans les fèces (entre 13 et 33 %). Les performances (gain moyen quotidien et indice de conversion alimentaire) ont aussi été améliorées par AFP. La résistance osseuse des porcs en croissance-finition a été très significativement et très fortement augmentée par AFP. © Inra/Elsevier, Paris

phytase / *Aspergillus fumigatus* / phosphore / digestibilité / performance / porc

1. INTRODUCTION

Diets for pigs and poultry are based mainly on ingredients of plant origin. In these feed components 75 % on average of the phosphorus (P) content is represented by phytic-P (*myo*-inositol-hexaphosphate). Phytic-P has a very low bioavailability for monogastric animals [32]. Therefore, the non-utilized P is excreted and can contribute to the P pollution problems in areas where animal production is intensive. Furthermore, phytate has inhibitory effects on the activity of several digestive enzymes [6, 33] and phytic acid complexes reduce protein digestibility [5]. Also, insoluble protein–metal-phytate complexes are formed below the isoelectric pH of proteins, reducing calcium (Ca), magnesium, iron and zinc absorption from the intestinal tract of monogastric animals and of humans [1, 33].

To utilize the P from phytate it must be hydrolysed by phytate degrading enzymes, such as phytase. The action of plant (EC 3.1.3.26) or microbial (EC 3.1.3.8) phytases on phytates may ameliorate antinutritive properties, increase available P and increase P retention by simple-stomached animals, as well as increase retention of Ca and micronutrients [15, 28, 30, 42]. Dietary microbial phytase supplementation can thus improve phytic-P digestive utilization and consequently reduce P pollution by animal excreta [17, 18, 23, 37, 38]. Furthermore, improved nutrient digestibility in animals fed supplemental phytase has been reported in pigs and in poultry [3, 4, 22, 24, 25, 46, 47].

However, phytases are very sensitive to the pelleting temperature commonly used for pig and poultry compound feed manufacture [17, 30, 39]. Thus, the challenge to produce a more thermostable phytase is very important, even if nowadays pelleting stability is to some extent improved by protected formulations that better resist pelleting.

Pure *Aspergillus fumigatus* phytase (AFP) has some interesting characteristics related to thermostability. The enzyme protein survives 20 min heating at 90 °C while phytases from *A. niger* and *A. terreus* are inactivated [44]. As other phytases, AFP is also unfolded after heating. However, only AFP has the capacity to refold completely into a native-like, fully active structure [44, 45]. The AFP gene has been cloned and overexpressed in *Hansenula polymorpha* [26]. Using phytic acid as a substrate, purified AFP showed activity between pH 2.5

and 8.0 with optima for pH values between 4.0 and 6.5 [26]. The aim of the present experiments was to evaluate the effects of AFP in vitro (in feed), and in vivo in the young and in the growing-finishing pig.

2. MATERIALS AND METHODS

2.1. Diets

Four experimental mash diets were used: two basal phytic-P-rich diets (A and C) and each of these diets supplemented with 500 U/ kg of the AFP liquid preparation (B and D). One unit of phytase is defined as the amount of enzyme which liberates 1 µmole of inorganic P per minute from 0.0015 mole per litre sodium phytate at pH 5.5 at 37 °C. The basal diets (*table I*) were formulated to meet the animals' requirement according to Henry et al. [16] and provided enough P without the addition of mineral P. They have also a very low endogenous phytase activity, 34 and 43 U/kg, respectively, for diets A and C. Four samples of each diet were analysed for dry matter (DM), nitrogen (N), Ca and P content as well as for phytase activity. DM and N were measured after excication during 24 h at 108 °C and by the Kjeldahl method, respectively. P determination was carried out colorimetrically by the vanadomolybdate procedure [2]. Ca content was determined with an atomic absorption spectrophotometer (Perkin Elmer, model Analyst 300, Norwalk, Connecticut, USA) [4]. The observed dietary levels of phytase were 490 ± 30 and 525 ± 28 U/kg for diets B and D, respectively.

2.2. In vitro evaluation

2.2.1. Release of P from the diets

Samples of all the experimental mash diets were used for this determination. Each diet was incubated at 37 °C, using adequate buffer solutions (devoid of P). They were first incubated at pH 2 (mimicking the gastric passage) for 1 h and then either at pH 5.5 (pH generally used for phytase activity determination) or at pH 7 (mimicking the pH conditions of the small intestine) for 4 h. The released P in the supernatant of the incubated material was measured by spectrophotometry in a micro-plates analyzer (ELX 808, OSI, F-78312 Maurepas). Each incubation protocol was repeated six times.

2.2.2. Determination of phytase resistance to pelleting

Samples of the phytase supplemented diets (B and D) were subjected to pelleting at increasing temperatures from 60 to 90 °C in steam-pelleting equipment (Bühler, Uzwil, Switzerland). The die used had the following characteristics: 4 mm of diameter and 25 mm of length. The programmed and the measured pelleting temperatures are presented in *table II*. The pelleted feed was then incubated at 37 °C, using adequate buffer solutions (devoid of P) for 6 h at pH 5.5 and the released P measured. Each incubation protocol was repeated six times.

2.3. In vivo evaluation

2.3.1. Apparent digestibility measurements

Eight growing pigs (Large-White × Landrace × Piétrain castrated males) with an average initial body weight of 42 ± 3.5 kg were used. Each animal was fitted with a permanent cannula in the brachiocephalic trunk as described by Simões Nunes et al. [41] allowing non-painful kinetic

Table I. Percentage composition of the basal diets A and C.

Ingredients	Diets	
	A[1]	C[2]
Maize	50	50
Rapeseed meal	35	30
Barley	12	17
Minerals[3], vitamins, aa	3	3
Phytic-P (%)	0.56	0.46
Phytase activity[4]	34	43

[1] Analysed content: crude protein (N × 6.25) = 18.1 %; lysine = 1.21 %; methionine + cystine = 0.75 %; Ca = 1.05 %; P = 0.73 %; estimated digestible energy = 14.21 MJ / kg.

[2] Analysed content: crude protein (N × 6.25) = 15.5 %; lysine = 1.04 %; methionine + cystine = 0.73 %; Ca = 0.96 %; P = 0.62 %; estimated digestible energy = 13.56 MJ / kg.

[3] Mixture without mineral phosphorus.

[4] U/kg; 1 U = 1 µmole of inorganic P liberated per min. from 0.0015 mole per liter sodium phytate at pH 5.5 at 37 °C.

blood withdrawal. After surgery the animals were individually housed in metabolic cages in an environmentally controlled room allowing easy access to the vascular cannulae as well as quantitative sampling of faeces. Room temperature was 21–22 °C and humidity percentage was 50 %. Their maintenance and that of the cannulae function were performed as described by Simões Nunes et al. [41]. Before surgery the animals were adapted for 10 days to the basal phytic-P diet (C). After surgery they were fed either this same diet (group C) or this diet supplemented with 500 U/kg of AFP (group D). The feed was distributed in mash form in two daily meals (0800 and 1500 hours) of 1 000 g each and the animals had free access to drinking water.

A multiple Latin square design protocol was used (*table III*). Throughout the experiment, blood was withdrawn twice a day (1000 and 1600 hours) for the determination of the P, Ca and alkaline phosphatase (ALP) levels. These parameters were measured according to Daly and Eninghausen [9], Gingler and King [12] and SFBC [43], respectively. Mean blood parameters were calculated for each of the 15-day periods. Faecal collection was performed from each animal during the last 5 days of each period. The fresh faeces were analysed for their DM, N, P and Ca content. The apparent digestibilities of DM, N, Ca and P were calculated. The digestibility of Ca was not corrected for Ca intake with the drinking water. Mean Ca content of the drinking water was 55 mg/L.

Table II. Pelleting conditions of the diets B and D used for the phytase resistance determination.

	Programmed temperatures (°C)			
	60	70	80	90
	Diet B			
1	55	65	77	84
2	67	68	79	83
	Diet D			
1	55	66	77	85
2	67	69	76	83

1, Temperature at the press; 2, temperature of delivered pellets.

Table III. Experimental design for the evaluation of the *Aspergillus fumigatus* phytase (AFP) preparation.

Animals	15-day period	15-day period
	Diet	
1, 3, 5 and 7	C	D
2, 4, 6 and 8	D	C

C, Basal diet; D, C supplemented with 500 U/kg of AFP.

2.3.2. Performance and biochemical evaluation in the piglet

Seventy-two weaned piglets (Large-White × Landrace × Piétrain castrated males) with an initial body weight of 9.5 ± 1.2 kg were used. They were allocated into two equal groups (A and B) and housed in cages in sub-groups of three animals, each in an environmentally controlled room. Room temperature was initially 27 °C and was lowered weekly by about 2 °C. Environment humidity percentage throughout the experiment was 50 %. The animals were fed for 28 days a basal phytic-P-rich diet (group A) or this diet supplemented with 500 U/kg of AFP (group B). The feed was distributed ad libitum in mash form and the animals had free access to drinking water.

Piglets were individually weighed weekly at the same time of the day during the 4-week study period. Cage feed intakes were recorded. Animal performance was evaluated for the 28-day observation period. Blood was collected, by jugular puncture, on day 28 from all the animals for the determination of the P, Ca and ALP concentrations.

Faecal P concentration was measured at the end of the observation period. Faeces were sampled by cage during the last 5 days of the trial.

2.3.3. Performance and biochemical evaluation in the growing-fattening pig

Sixty-four growing pigs (Large-White × Landrace × Piétrain castrated males) with an initial body weight of 35 ± 4 kg were used. For 10 days all the animals received the phytic-P-rich diet C. After that period the animals were allocated to two equal groups (C and D) and housed in floor-pen cages in sub-groups of four animals each in an environmentally controlled room. Room tem-

perature was 21–22 °C and humidity percentage was 50 %. The pigs were fed the basal phytic-P-rich diet (group C) or this diet supplemented with 500 U/kg of AFP (group D) for 59 days. The feed was distributed ad libitum in mash form and the animals had free access to drinking water.

Animal performance was evaluated after 36 days and for the 59 days of trial duration. Blood was collected, by jugular puncture, on days 0, 19, 36 and 56 from all the animals for the determination of the plasma P, Ca and ALP levels.

Faecal P concentration was measured on days 31 and 59. Faeces were sampled by cage during the last 5 days preceding these dates.

At the end of the evaluation eight animals from each of the experimental groups (one from each pen) were slaughtered after electronarcosis for bone collection. The collected bones were the right femur, tibia, humerus as well as the main external metacarpal and metatarsal. Samples were prepared from each of the collected bones immediately after slaughter. After careful dissection and removal of the soft tissue, a diaphysis section was obtained by sawing each bone. The sections obtained were about 8 cm long for the femur, tibia and humerus and about 3 cm for the main external metacarpal and metatarsal bones. The obtained bone sections were immediately subjected to compression in order to determine the force in Newton necessary to break them (maximal breaking force at the fracture point). The measurement was performed with a LR10K compression machine, using a XLC/10K/-A1 force captor and a compression device TH23-196/AL (Lloyd Instruments, Fareham, UK).

2.4. Statistical analysis

Statistical treatment of the results involved the calculation of the mean and of the standard deviation of the mean as well as an analysis of variance followed by a Duncan test [35].

3. RESULTS

3.1. In vitro release of P and determination of phytase resistance to pelleting

The results obtained for the measurement of in vitro release of P from the mash diets are presented in *table IV*. After incubation at pH 5.5, the P liberated in the phytase-supplemented feed represented 305 and 327 % of that liberated by the control feed, respectively, for diets B and D. After incubation at pH 7, the respective results were 330 and 282 %, but the amount of liberated P was lower compared with pH 5.5. Thus, feed supplementation with *A. fumigatus* phytase resulted in an in vitro P liberation about three times higher than that obtained from

Table IV. Effects of *A. spergillus fumigatus* phytase (AFP) on the in vitro in feed inorganic phosphorus liberation.

Parameters	Diets			
	A	B	C	D
Incubation: 1 h at pH 2 followed by 4 h at pH 5.5				
µmole of P/g of feed	61.9 ± 3.10[1,a]	188.9 ± 11.34[b]	80.5 ± 1.61[a]	263.3 ± 11.06[b]
	(100 %)	(305 %)	(100 %)	(327 %)
Incubation: 1 h at pH 2 followed by 4 h at pH 7.0				
µmole of P/g of feed	40.3 ± 8.46[a]	133.2 ± 9.32[b]	52.7 ± 4.21[a]	148.7 ± 16.35[b]
	(100 %)	(330 %)	(100 %)	(282 %)

A and C, basal diets; B and D, A and C supplemented, respectively, with 500 U/kg of ATP.
[1] Mean ± standard deviation of the mean of six determinations.
[a, b] For each pair of diets, values with different superscript letters are statistically different $P < 0.001$.

the basal diet, irrespective of the pH value used for the determination.

When the feed was pelleted at a temperature higher than 70 °C, a reduction in P liberated was observed (*table V*). For an effective pelleting temperature of about 84 °C (*table II*), the free P obtained after incubation represented for both diets slightly more than 50 % of that obtained from the corresponding mash feed (*table V*).

3.2. Effects of *A. fumigatus* phytase on performance

In both piglets and growing-finishing pigs, AFP increased the average daily gain and decreased the feed conversion ratio (*tables VII* and *VIII*). The intensity of the effects was statistically significant in piglets and during the first 36 days of observation in growing-fattening animals.

Table V. Effects of the pelleting temperature on the in vitro phosphorus liberation (µmol/g) from the diets B and D supplemented with 500 U/kg of *Aspergillus fumigatus* phytase. Incubation for 6 h at pH 5.5

Temperature of pelleting (°C)	µmole of P liberated per g	
	Diet B[1]	Diet D[2]
Mash form diet	167.7 ± 18.09[3] (100 %)	182.8 ± 10.50 (100 %)
60°	167.8 ± 13.11 (100 %)	176.0 ± 15.07 (96 %)
70°	161.6 ± 9.37 (96 %)	152.3 ± 12.04 (83 %)
80°	112.2 ± 15.48 (67 %)	121.5 ± 8.78 (66 %)
90°	87.2 ± 5.66 (52 %)	98.7 ± 14.99 (54 %)

[1] Mean free inorganic phosphorus concentration 34.7 ± 2.55 µ mole/g.

[2] Mean free inorganic phosphorus concentration 43.6 ± 2.89 µ mole/g.

[3] Mean ± standard deviation of the mean of six determinations.

3.3. Influence of *A. fumigatus* phytase on blood biochemistry

In all three in vivo experiments, AFP supplements had clear and significant effects on the plasma levels of P, ALP and Ca. Mean phosphataemia was increased and mean phosphatasaemia and calcaemia were decreased in the pigs fed diets B and D (*tables VI, VII* and *IX*). In growing-fattening animals, the degree of the effects was more pronounced during the first 36 days of the observation period than in the later phase. There was no significant difference between the calcaemia of these animals at the end of the experiment (*table IX*).

3.4. Effects of *A. fumigatus* phytase on digestibility and on faecal P concentration

The apparent digestibilities of DM and N were not modified by AFP. P digestibility was significantly higher for the diet supplemented with AFP (*table VI*). The improvement represented 22 percentage units which is equal to 1.37 g digestible P per kg of diet. The increase in the Ca digestibility (3.9 percentage units) as a result of AFP was not statistically significant.

In all three experiments, AFP supplementation significantly decreased P concentration in faeces. In the catheterized animals, the P content of the AFP-treated animals was 33 % lower than in the controls (*table VI*). In piglets, the reduction was only 13 % (*table VII*) and, in the growing-fattening pigs this was of 18 % (*table VIII*), irrespective of the period of measurement (27–31 or 55–59 days).

3.5. Effects of *A. fumigatus* phytase on bone strength

Breaking force of the humerus, femur, tibia, main external metatarsal and main external metacarpal bones was significantly

Table VI. Effects of *Aspergillus fumigatus* phytase (AFP) on phosphataemia, calcaemia, phosphatasaemia, P faecal concentration (PFC), and dry matter (DM), nitrogen (N), P and Ca digestibilities (% of the intake) in the catheterized growing pig.

Parameters	C	D
Phosphataemia (mg/dL)	$6.66 \pm 0.69^{1,a}$	8.33 ± 0.93^{b}
Phosphatasaemia (U/L)	196 ± 36^{c}	158 ± 33^{d}
Calcaemia (mg/dL)	11.9 ± 0.91^{e}	10.6 ± 0.77^{f}
PFC (% of DM)	$2.83 \pm 0.19^{2,a}$	1.89 ± 0.18^{b}
Digestibility DM	83.2 ± 1.4^{2}	84.3 ± 1.2
N	81.8 ± 0.37	82.1 ± 0.42
P	30.8 ± 2.03^{a}	52.8 ± 1.87^{b}
Ca	43.3 ± 3.61	47.2 ± 2.61

C, basal diet; D, C supplemented with 500 U/kg of AFP.
[1] Mean ± standard deviation of the mean 164 determinations; [2] mean ± standard deviation of the mean of 40 determinations.
Feed determined phytase activity: C, 43 U/kg; D, 525 U/kg.
[a, b, c] Values with different superscript letters are significantly different: [a, b] $P < 0.001$; [c, d] $P < 0.01$; [e, f] $P < 0.05$.

Table VII. Effects of *Aspergillus fumigatus* phytase (AFP) dietary supplementation on average daily gain (ADG), feed conversion ratio (FCR), phosphataemia, calcaemia, phosphatasaemia and P faecal concentration (PFC) in the piglet.

Parameters and periods	A	B
ADG (g)	$301 \pm 55^{1,a}$	341 ± 43^{b}
FCR (kg/kg)	$1.82 \pm 0.09^{2,a}$	1.62 ± 0.07^{b}
Phosphataemia (mg/dL)	$4.05 \pm 0.50^{3,a}$	6.21 ± 0.94^{c}
Phosphatasaemia (U/L)	$214 \pm 30^{3,a}$	156 ± 37^{b}
Calcaemia (mg/dL)	$9.52 \pm 0.76^{3,a}$	8.35 ± 0.87^{b}
PFC (% of DM)	$2.21 \pm 0.07^{4,c}$	1.92 ± 0.06^{d}

A, basal diet; B, A supplemented with 500 U/kg of AFP.
Diet based on: maize, rapeseed and barley, without any addition of mineral or organic phosphorus.
Feed determined phytase activity: A, 34 U/kg; B, 490 U/ kg.
Animals: piglets of an initial body weight of 9.5 ± 1.2 kg.
[1] Mean ± standard deviation of the mean of 36 determinations; [2] mean ± standard deviation of the mean of 12 replicates; [3] mean ± standard deviation of the mean of 36 – day 28; [4] mean ± standard deviation of the mean of 12 replicates - day 24 to day 28.
[a, b, c] Values with different superscript letters are significantly different: [a, b] $P < 0.05$; [c, d] $P < 0.01$.

higher in the animals fed on the diet supplemented with the AFP preparation (*table X*). The effect was particularly intense on the metacarpal (+119 %), tibia (+141 %) and metatarsal (+227 %).

4. DISCUSSION

One of the very important problems in the utilization of enzymes as feed supplements is their susceptibility to resist to the conditions used in the manufacture of com-

Table VIII. Effects of *Aspergillus fumigatus* phytase (AFP) dietary supplementation on average daily gain (ADG), feed conversion ratio (FCR) and P faecal concentration (PFC) in the growing pig.

Parameters	Period (days)	Diets	
		C	D
ADG (g/day)	0–36	928 ± 54[1, a]	1 019 ± 78[b]
	36–59	747 ± 65	787 ± 81
	0–59	873 ± 51	913 ± 73
FCR (kg/kg)	0–36	2.42 ± 0.08[2, a]	2.27 ± 0.12[b]
	36–59	3.45 ± 0.12	3.25 ± 0.17
	0–59	2.72 ± 0.13	2.64 ± 0.18
PFC (% of DM)	27–31	2.48 ± 0.09[2, c]	2.03 ± 0.06[d]
	55–59	2.47 ± 0.11[c]	2.02 ± 0.07[d]

C, Basal diet; D, C supplemented with 500 U/kg of AFP.
Diet based on: maize, rapeseed and barley, without any addition of mineral or organic phosphorus.
Feed determined phytase activity: C, 43 U/kg; D, 525 U/kg.
Animals: growing pigs of an initial body weight of 35 ± 4 kg.
[1] Mean ± standard deviationof the mean of 32 determinations; [2] mean ± standard deviationof the mean of eight replicates.
[a, b, c] Values with different superscript letters are significantly different: [a, b] $P < 0.05$; [c, d] $P < 0.01$.

Table IX. Effects of *Aspergillus fumigatus* phytase (AFP) dietary supplementation on phosphataemia, phosphatasaemia and calcaemia in the growing pig.

Period (day)	Phosphataemia (mg/dL)		Phosphatasaemia (U/L)		Calcaemia (mg/dL)	
	Diets					
	C	D	C	D	C	D
0	5.23 ± 1.07[1]		219 ± 40		11.8 ± 0.54	
19	5.1 ± 0.71[2, a]	8.7±1.2[b]	244 ± 52[a]	169 ± 27[b]	12.9 ± 0.38[c]	10.3 ± 0.27[d]
36	6.1 ± 0.63[a]	7.7±0.49[b]	229 ± 36[c]	172 ± 27[d]	12.4 ± 0.46[c]	9.9 ± 0.52[d]
56	6.5 ± 1.21[e]	7.8±0.65[f]	208 ± 39[e]	164 ± 27[f]	11.4 ± 0.61	10.9 ± 0.44

C, Basal diet; D, C supplemented with 500 U/kg of AFP.
Diet based on: maize, rapeseed and barley, without any addition of mineral or organic phosphorus.
Feed determined phytase activity: C, 42.8 U/kg; D, 525 U/ kg.
Animals: growing pigs of an initial body weight of 35 ± 4 kg.
[1] Mean ± standard deviation of the mean of 64 determinations; [2] mean ± standard deviation of the mean of 32 determinations.
[a, b, c] Values with different superscript letters are significantly different: [a, b] $P < 0.001$; [c, d] $P < 0.01$; [e, f] $P < 0.05$.

pound feed. Phytase is particularly sensitive to steam-pelleting [17, 30, 38]. It has been demonstrated that pure AFP has the ability to refold completely into a native-like, fully active structure after heat denaturation at up to 90 °C [45]. Purified AFP has a much higher temperature resistance than purified *A. niger* phytase [44]. Wild-

Table X. Effects of *Aspergillus fumigatus* phytase (AFP) dietary supplementation on bone strength (Newton/m at the breaking-down point) in the growing-fattening pig.

Bone section	Diet	
Parameters	C	D
Humerus	2 186 ± 156[1, a]	3 210 ± 228[b]
Femur	1 352 ± 321[a]	2 293 ± 265[b]
Tibia	907 ± 103[a]	2 187 ± 215[b]
Main external metatarsal	161 ± 67[a]	526 ± 79[b]
Main external metacarpal	210 ± 75[a]	461 ± 86[b]

C, Basal diet; D, C supplemented with 500 U/kg of AFP.
Diet based on: maize, rapeseed and barley, without any addition of mineral or organic phosphorus.
Feed determined phytase activity: C, 42.8 U/kg; D, 525 U/ kg.
Animals: growing pigs of a final body weight of 82 ± 6 kg.
[1] Mean ± standard deviation of the mean of eight determinations.
[a, b] Values with different superscript letters are significantly different: $P < 0.001$.

type *A. niger* phytase [38] and vegetable phytase [30] lost about 80 % of their activity when the feed was pelleted at about 80 °C. AFP was therefore, much more resistant to increased pelleting temperature. However, when the effective pelleting temperature reaches 83–85 °C the remaining activity, as assessed by the in vitro in feed P liberation, is about 50 % of the added enzyme. The thermal resistance of the protein after incorporation in the feed appeared to be negatively influenced during the pelleting process either by the duration of exposure to a high temperature and pressure or by interaction with the dietary components. Thus, the improved temperature resistance of AFP which is sufficient for a good retention of activity when the feed was pelleted at temperatures up to 70–80 °C was not effective when pelleting feed at higher temperatures (up to 90 °C).

The assessment of P availability is difficult to perform correctly [10, 21, 34]. Moreover, in the pig, and in contrast to poultry, the effects of low P bioavailability on bone parameters appear only after a relatively long time (more than 1 month) after ingestion of diets severely deficient in P. Conversely, phosphataemia of both species is a very sensitive parameter of the P bioavailability in the diet [29, 37]. For this reason, kinetic phosphataemia was used as one of the evaluation criteria together with faecal P digestibility and excretion and the evaluation of classical performance parameters. The consumption of the phytate-rich diets systematically induced hypophosphataemia. Normal blood P level was restored by AFP even during the finishing phase. Often in the finishing period, phosphataemia of non-supplemented animals appears within the physiological values [37]. This improvement could be related to a lower P need combined with better digestive utilization of phytic-P observed in finishing pigs [19, 20, 32, 37]. In the present study the effects of the phytase preparation on phosphataemia of growing-fattening pigs were more pronounced during the first 19 days than later in the observation period.

Mean calcaemia of the non-supplemented animals was generally higher than the normal range of pig values. The high plasma values were reduced by the addition of AFP to the diet. It should be emphasized that, in severe P dietary deficiency, hypercalcaemia is associated with hypophosphataemia in both pigs and poultry [7, 27, 29, 30, 36]. In the short-term, calcium blood level caused by low P bioavailability is often within the normal values [37]. Phosphatasaemia of the

AFP groups was lower than that of the controls. The presence of elevated plasma ALP concentration in pigs fed phytic-P-rich diets is thus confirmed by the present study [11, 29, 37].

It is now generally accepted that active phytase preparations can reduce faecal P excretion into the environment by improvement of phytic-P bioavailability [8, 14, 15, 31, 34]. The AFP included in the diet at a level of 500 U/kg strongly increased the P digestibility of growing pigs by 22 percentage units and accordingly reduced the P excreta load. This AFP effect appears to be higher than that of *A. niger* phytase in experiments performed under the same conditions [40].

In the present study Ca digestibility was improved slightly by 3.9 percentage units by the addition of AFP to the diet. Effects of phytases on Ca digestibility are contradictory. Ca digestibility in pigs has been shown to be significantly improved [1, 19] or not [13, 14] by phytase preparations. AFP did not have significant effects on the DM and N faecal digestibilities. The effects of AFP on amino acid availability remain to be investigated by measurement of either ileal digestibility [24] or the gastrointestinal absorption of amino acids, in order to overcome the effects of the hindgut microflora on the amino acids availability.

AFP significantly influenced bone strength. This observation confirms the effects of phytases on the bone resistance reported by other authors [1, 8, 13, 22, 29, 30].

In conclusion AFP is very efficient in the pig and it represents a major step in the development of a heat-stable phytase.

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