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The impact of microbial phytase on the nutrition of monogastrics and the environment

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SUMMARY - In this paper the current status of knowledge on the impact of microbial phytases in pig and poultry production is reviewed, in relation to the environment. The emphasis is put on the research on pigs, Firstly, information is given on the impact of legislation in the Netherlands, which focuses on P and N, with regard to livestock production and the environment. The mode of action of extrinsic phytases and an analytical method to measure their activity are reviewed. Supplementation of commercially available microbial phytases to diets for pigs and poultry results in enhancement of P digestibility/availability, as assessed by different criteria. In studies with pigs at our institute, the doses of microbial phytase (Natuphos®) in the range of 500 to 2000 phytase units per kg of feed resulted in generation of 0.8 to 1.0 g digestible P per kg of feed. The dose-response relationship seems to be dependent on the type of feed and the dosis of microbial phytase. In addition, the content of digestible calcium is increased, and amounts on average from 50 to 80% of the increase of digestible P content. Efficacy of microbial phytases seems to be different for particular categories of pigs (piglets, growing pigs, breeding sows during pregnancy or lactation) and poultry (broilers or laying hens). Feeding frequency and amount of feed exert minor effects on the efficacy of microbial phytase. In contrast, soaking a phytate-rich diet or addition of lactic or formic acid to a diet with microbial phytase from Natuphos® had a synergistic effect on the apparent digestibility of P and calcium. The amount and origin of phytate appears to have a substantial effect on the efficacy of microbial phytase. Up to 1.2 g of phytate P per kg of feed is limiting for a maximal effect of 450 phytase units per kg of feed. Apart from the well-defined positive effects of this enzyme on calcium and P digestibility, there are also some indications that the ileal digestibility of amino acids, and the total tract digestibility of magnesium and trace elements (zinc and copper) are enhanced by microbial phytase. Performance (daily gain, feed conversion ratio) of pigs fed microbial phytase appears to be better as compared with a non-supplemented diet or with a positive control diet. Microbial phytase supplementation to diets for pigs and poultry can reduce substantially P excretion and, therefore, reduce environmental pollution.

Key words: Pigs, poultry, microbial phytase, phosphorus, calcium, digestibility, performance, environment.

RESUME - *"L'impact de la phytase microbienne sur la nutrition chez les monogastriques et sur l'environnement". Cet article passe en revue l'état actuel des connaissances concernant l'impact sur l'environnement des phytases microbiennes en production avicole et porcine, en insistant sur la recherche menée en production porcine. En premier lieu, des informations sont présentées sur l'impact de la législation des Pays-Bas, axée sur le P et N, en ce qui concerne la production animale et l'environnement. Le mode d'action des phytases extrinsèques est décrit ainsi qu'une méthode analytique pour mesurer leur activité. La supplémentation en phytases microbiennes disponibles dans le commerce pour des régimes porcins et avicoles donne comme résultat l'augmentation de la digestibilité/disponibilité en P, tel qu'on l'a évalué d'après différents critères. Dans des études menées sur des porcins à notre Institut, les doses de phytase microbienne (Natuphos®) dans un spectre de 500 à 2 000 unités de phytase par kg d'aliment ont donné lieu à 0,8 jusqu'à 1,0 g de P digestible par kg d'aliment. Le rapport dose-réponse semble être sous la dépendance du type d'aliment et de la dose de phytase microbienne. En outre, la teneur en calcium digestible est augmentée, et représente en moyenne de 50 à 80% de l'augmentation de teneur en P digestible. L'efficacité des phytases microbiennes semble être différente pour des catégories particulières de porcins (porcelets, porcs en croissance, truies en reproduction pendant la gestation ou la lactation) et de volailles (poulets de chair et poules pondeuses). La fréquence des prises alimentaires et la quantité d'aliment ont des effets moindres sur l'efficacité de la phytase microbienne. Par contre, le fait de faire tremper un régime riche en phytates, ou l'addition d'acide lactique ou formique à un régime contenant des phytases microbiennes avait, selon Natuphos®, un effet de synergie sur la digestibilité apparente du P et du calcium. La quantité et l'origine des phytates semble avoir un effet important sur l'efficacité de la phytase microbienne. Lorsque l'on atteint 1,2 g de P provenant de phytates par kg d'aliment, ceci est limitant pour un effet maximum des 450 unités de phytase par kg d'aliment. Outre les effets positifs bien connus de cette enzyme sur* *la digestibilité du calcium et du P, il y a encore quelques indices montrant que la digestibilité iléale des acides aminés, et la digestibilité totale dans le tractus du magnésium et des oligoéléments (zinc et cuivre) sont augmentés par la phytase microbienne. Les performances (gain moyen quotidien, indice de conversion alimentaire) des porcins recevant une phytase microbienne semblent être meilleures que pour ceux recevant un régime non supplémenté ou ayant un régime témoin positif. La supplémentation des régimes en phytase microbienne pour les porcins et volailles peut réduire de façon substantielle l'excrétion de P et, par conséquent, réduire la pollution environnementale.*

Mots-clés : *Porcins, volailles, phytase microbienne, phosphore, calcium, digestibilité, performances, environnement.*

Introduction

Plant ingredients used to formulate diets for pigs and poultry may contain from 0.7 to 3.5% of phytates (myo-inositol hexakisphosphates) in a form of poorly soluble Ca-Mg, K-Mg, or mono-ferric and zinc salts of phytic acid (Cosgrove, 1980; Maga, 1982; Suttle, 1983). The digestibili ty/availability of these salts for monogastrics is very limited (Cromwell, 1980; Jongbloed, 1987; Simons *et al.*, 1992), and they will predominantly be excreted. Phytates, therefore, contribute to the phosphorus (P) pollution in regions where land and water resources are limited and animal production is intensive like in the Netherlands. Theoretically, the amount of P present in plant phytates should be sufficient to meet the P requirements of pigs and poultry. In many commonly used feedstuffs (maize and maize-by-products, soybean meal, rapeseed meal), the proportion of phytate-bound P to total P is high, but they lack intrinsic phytases, enzymes that cleave the ortho-phosphate groups from the phytate molecule. To enable dephosphorylation of the dietary phytates, there is an increasing interest all over the world in supplementing such diets with purified preparations of extrinsic (microbial) phytases, instead of additional P from feed phosphates. These phytases are often obtained from genetically modified micro-organisms.

In this paper, the effects of extrinsic phytases in feeds for pigs and poultry on the availability of P, some other minerals and N, and on performance will be discussed. Furthermore, attention is paid to dietary and management factors that affect efficacy of microbial phytase. Emphasis will be put on the research with pigs. Also, attention will be paid to environmental regulations to reduce P load on the soil, particularly in the Netherlands.

Legislation in the Netherlands with regard to livestock production and P pollution of the environment

In 1984 new legislation was enforced in the Netherlands. Global aims of governmental policy are: (i) equilibrium fertilization; (ii)) reduction of acid deposition; (iii) protection of surface and ground water quality. Apart from the pollution with P, reduction of N output by animal production into the environment becomes more and more important. P content of the ground and surface water should not exceed 0.10 mg $P_2O_5.l^{-1}$ (about 0.15 mg $P_t.l^{-1}$). Legislation firstly concentrated on reduction of application of P per hectare of land. The need may be illustrated by the fact that in animal dense areas in the southern and eastern part of the Netherlands between 200 and 250 kg $P_2O_5.ha^{-1}$ was applied from animal manure, while withdrawal by crops is on average 50 kg $P_2O_5.ha^{-1}$ (Jongbloed and Henkens, 1996). Thus, high accumulation of P took place per hectare of cultivated land, which is not acceptable. The amount of animal manure that can be applied per ha of land is based on its P content (Table 1). If the P_2O_5 production on a farm exceeds the amount allowed, the farmer has to pay a fine for the surplus, which has to be transported to other regions. Manure transport can be facilitated by the manure bank. If the P_2O_5 or NH_3 goals cannot be achieved, finally the number of animals must be reduced. So far, it has been shown in the Netherlands, that nutrition has been most successful in reduction of N and P excretion, a.o. by phase feeding and the use of microbial phytase (Jongbloed and Henkens, 1996).

Table 1. Allowed application of P_2O_5 (kg.ha^{-1}) from 1987 onwards in the Netherlands

	Arable land	Grassland	Maize land
≤1990	125	250	350
<1994	125	200	150
1995	110	150	110
1998	100	120	100
2000	85	85	85
≥2002	80	80	80

Some characteristics of microbial phytases

There are several microbial phytases commercially available, either as a dry powder or as a liquid. The phytases may slightly differ from each other in their physical properties and enzyme activity. Natuphos® was the first commercially available phytase from a genetically modified *Aspergillus niger* strain. Therefore, it is obvious that most knowledge concerning microbial phytase has been obtained with this enzyme. Its phytase activity (FTU) in the diets is as sayed by measuring the amount of ortho-phos- phates released from phytic acid within a period of linear increase with time. One unit of phytase activity is equal to 1 µmol of ortho-phosphate liberated from 1.5 mmol of Na-phytate within 1 min. at 37°C and pH 5.5 (Engelen *et al.,* 1994). The activity of some other phytases are assayed under slightly different conditions. The mode of action of phytase is illustrated in Fig. 1.

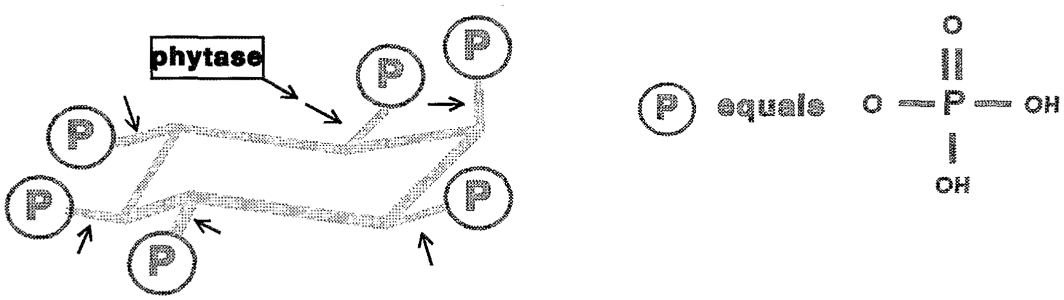

Fig. 1. Schematic mode of action of microbial phytase on dietary phytates.

The enzymes cannot stand high temperatures. For instance, by pelleting a pig diet at 70 °C, the initial activity is reduced by 15-25% (Schwarz and Hoppe, 1992). Microbial phytase is active over a wide pH range, with optima at pH 2.5 and 5.5 (Simons *et al.,* 1990). Jongbloed *et al.* (1992) reported from studies on cannulated pigs (*ileum+duodenum*) that hydrolysis of phytate in diets by microbial phytase takes place mainly in the stomach (43%), and decisively less in the small intestine (7%). Irrespective of the diet, no phytase activity could be detected in the ileal digesta of pigs. Moreover, no anatomo-pathological changes were detected in some organs (liver, kidneys and heart) of pigs fed microbial phytase (Beers and Jongbloed, 1992b).

The effect of microbial phytase on digestibility/availability of P

Since 1990, several experiments with exogenous microbial phytases were reported to quantify their effect on the apparent digestibility/availability of P. A survey of a large part of these studies has been presented by Jongbloed *et al.* (1993) and Düngelhoef and Rodehutscord (1995). The effect of microbial phytase (Natuphos®) on the apparent digestibility of P was investigated in a dose-response experiment on growing pigs from 20 to 55 kg body weight (Beers and Jongbloed, 1992a). Six doses of phytase (from 0 to 2000 FTU.kg^{-1}) were used in two types of grower diets (based either on maize-soybean meal or phytate-rich by-products). The efficacy of microbial phytase appeared to be related to its dose and the type of diet (Fig. 2). For the maize-soybean meal based diet the relation could be illustrated by an exponential curve, with the following formula:

$$digestible\ P\ (g.kg^{-1}) = 1.86 - 0.9963^{dose}$$

R^2 = 96.7%; r.s.d. = 0.067 g dig.P.kg^{-1}

From 0 to 400 FTU.kg^{-1} there was a rapid increase in microbial phytase efficacy, which was flattened afterwards. For the second type of diet, the response could be illustrated by a logistic curve, with the following formula:

$$digestible\ P\ (g.kg^{-1}) = 0.95 + \frac{1.31}{(1 + e^{(-5.51 \times 10^{-3} \times (dose - 377.8))})}$$

R^2 = 95.5%; r.s.d. = 0.092 g dig.P.kg^{-1}

From this experiment it was concluded that the efficacy of microbial phytase per FTU appeared to be the largest up to 500 FTU/kg diet. This dose was estimated to be equivalent to 0.8 g digestible P/kg diet.

Based on the curve for the maize-soybean meal based diet (Fig. 2) the responses of a large number of our own experiments and those of the literature for these types of diets were plotted (Fig. 3). Only those experiments were included that fulfilled several specific criteria. Figure 3 shows that the response of the other experiments at our institute fit quite well with the curve for maize-soybean meal. However, agreement of literature data with our dose-response curve is less well, which may be attributed by different procedures used. In all experiments it is shown that microbial phytase is effective in enhancing P digestibility considerably, and it thereby increases the amount of digestible/available P in the feed for pigs.

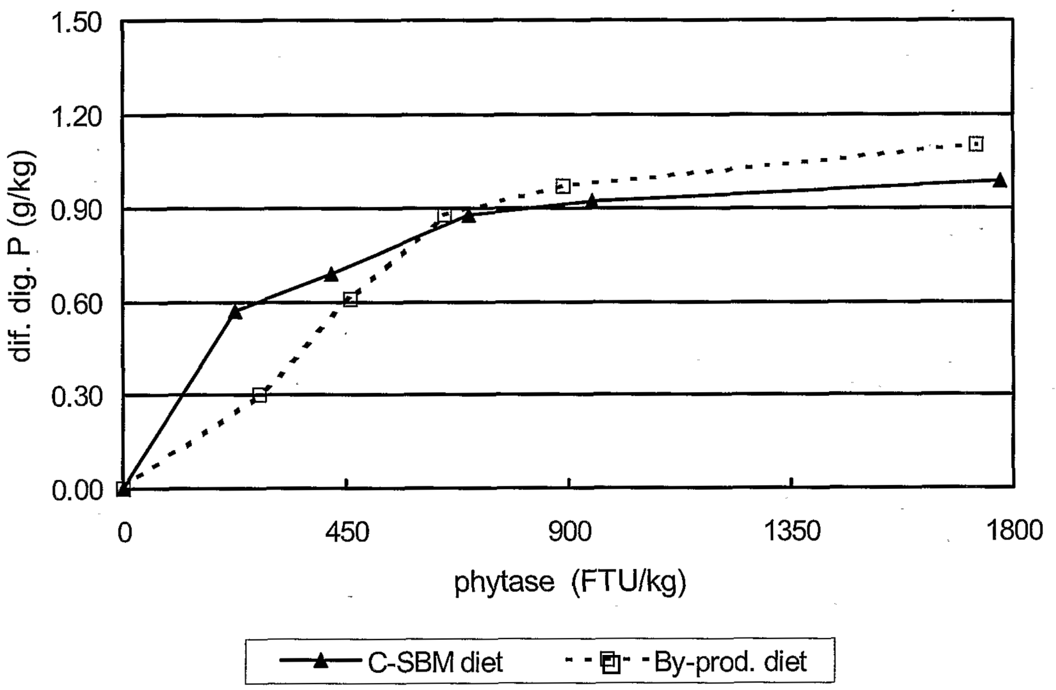

Fig. 2. Improvement of digestible P by microbial phytase (Natuphos ®) in two diets for growing pigs. (Beers and Jongbloed, 1992a)

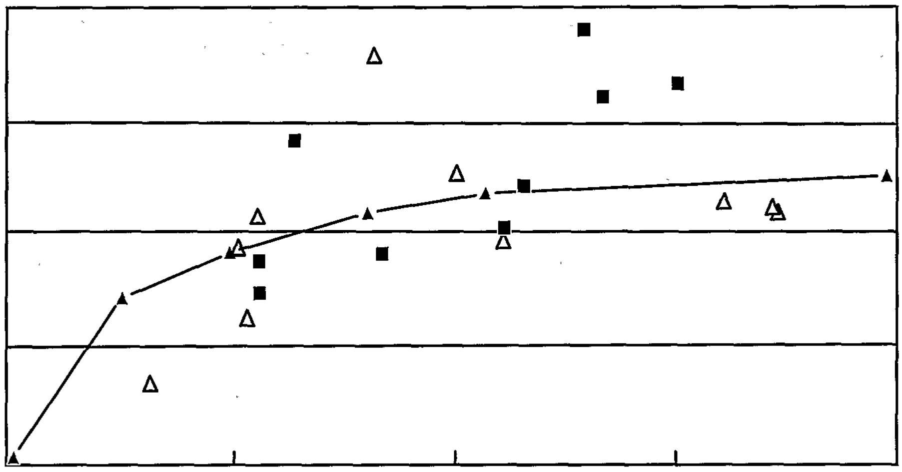

Fig. 3. Improvement of digestible P (g/kg) by microbial phytase in diets for pigs. (own experiments and literature)

Efficacy of microbial phytase in broilers is slightly lower than in pigs. Based on P retention, Schöner *et al.* (1991, 1993) calculated equivalencies ranging from 700 to 1050 FTU/kg for 1 g of P from monocalcium phosphate (MCP) (equal to 0.84 g available P), dependant on age/live-weight of the animal. Simons and Versteegh (1993) found that 500 FTU was equal to 1 g of P from MCP, based on P retention and a decline in yield per FTU at higher doses. Yi (1995) found equivalencies ranging from 850 to 1240 FTU for 1 g P from MCP, based on toe ash contents and BW gain (recalculated, using equivalencies between MCP and defluorinated phosphate according to Parr (1996)). Information about efficacy of microbial phytase in laying hens is very limited. Simons and Versteegh (1993) showed a minimal equivalency of 0.3 g P from MCP per 100 FTU up to 300 FTU/kg feed, which was based on performance. Van der Klis *et al.* (1992), however, found that 250 FTU was equal to 1.2 g P from MCP, based on ileal P absorption in a diet containing 30 g $Ca.kg^{-1}$. Using a diet containing 35 g $Ca.kg^{-1}$, Van der Klis *et al.* (1996) observed an equivalency of 0.8 g MCP per 250 FTU. From these figures it can be concluded that microbial phytase efficacy is superior in laying hens compared to broilers.

Body weight and physiological status of pigs seem to influence digestion and absorption of dietary phytates, although information on these aspects is scarce. Kemme *et al.* (1996) studied the digestibility of P and the efficacy of microbial phytase (500 FTU. kg^{-1}) in piglets, growing-finishing pigs and multiparous sows. They used an identical diet composed of maize, tapioca, phytase-inactivated wheat bran, peas, soybean meal and sunflowerseed meal. A summary of the results is presented in Table 2. The largest effect of microbial phytase on generating digestible P was noted for lactating sows, and the lowest during mid pregnancy. This lower value during pregnancy is due to the lower requirement of digestible P. The generation of digestible P by piglets and growing pigs was found to be between these extremes. Piglets had somewhat lower improvement in P digestibility than growing pigs.

Table 2. Effect of microbial phytase in relation to body weight and physiological status of swine on increase of P digestibility (%) and digestible P (g/kg)

Pig category	Piglets	Grower	Multiparous sows		
		Finisher Lactation	Pregnancy		
Weight (kg)	30-38	40-100	Middle	End	
P digestibility	13.6	17.2	6.9	15.2	21.5
Digestible P	0.66	0.84	0.32	0.72	1.04

Dietary and management factors that affect efficacy of microbial phytase

A prerequisite for a correct evaluation of the efficacy of microbial phytase is that the animals are fed below their P requirement, due to intestinal regulation of the absorption of P when animals are fed above P requirement. Studies in which the animal's P requirement is widely exceeded, can therefore not be used for this purpose.

There is little information available of dietary factors that affect efficacy of microbial phytase. It is commonly known that higher dietary Ca levels decrease apparent absorption of P (Jongbloed, 1987). In most experiments only in diets with microbial phytase different Ca levels were implemented and not in diets without microbial phytase at the same time (Lei *et al.,* 1994; Lantzsch *et al.,* 1995). Mostly, a linear decrease of P absorption is observed. In two experiments the potential interaction was studied between dietary Ca levels on P absorption in diets with and without supplementary microbial phytase (Mroz *et al.*, 1994b; Jongbloed *et al.,* 1995b). The apparent digestibility of P linearly decreased at higher Ca levels, but no interaction with microbial phytase could be demonstrated. This was confirmed by Kornegay (1995) in broilers and by Van der Klis *et al.* (1992) in layers.

Another dietary factor is the amount and source of phytate. In two experiments with pigs we studied the effect of the amount and source of phytate on the efficacy of microbial phytase (Dekker *et al.,* 1992). A diet based on maize or sunflowerseed meal at two inclusion levels (two levels of phytate P) was used. The concentration of phytate P was 1.2 and 1.8 g/kg in both diets and phytase activity in the maize and sunflower based diets were 450 and 340 FTU/kg, respectively. From the response on P digestibility it was concluded that in both diets the lower level of phytate was too low to get maximal effect of the enzyme, and that phytate in maize is more readily available than phytate in sunflower seed meal. Lack of substrate (phytate) may occur in piglet diets formulated with large proportions of animal products as a protein or P source or with diets that already contain a high concentration of intrinsic phytase (Eeckhout and De Paepe, 1992b; Düngelhoef *et al.,* 1994).

Jongbloed *et al.* (1995a) performed an experiment with pigs in which diets were either or not supplemented with microbial phytase and/or 3% lactic acid. Supplementary lactic acid exerted a synergistic effect on apparent digestibility of P. This means that efficacy of microbial phytase could be further enhanced by lactic acid. In a second experiment Jongbloed and Jongbloed (1996) confirmed this synergistic effect for formic acid, but not for lactic acid.

The efficacy of microbial phytase (500 $FTU.kg^{-1}$) could be increased by soaking a diet deficient in intrinsic phytase (maizé, tapioca, beans, phytase-inactivated wheat bran and extracted sunflower meal) prior to feeding for 8-15 hours (Kemme and Jongbloed, 1993). Soaking had no influence on P digestibility of the phytase-deficient diet, and P digestibility was increased by 18% when phytase was supplemented to the diet. When the phytase-supplemented diet was soaked, P digestibility was increased by 26% as compared to the non-soaked phytase-deficient diet. Näsi and Helander (1994) soaked a barley-soybean meal diet supplemented with microbial phytase for three hours, but it did not result in a higher P digestibility. In a second experiment of Näsi *et al.* (1995) soaking a barley-rapeseed meal diet for three hours with dried whey at 40°C resulted in a 4% increase of P digestibility. Maybe the short soaking time and the rather high dietary Ca content are due to this diminished effect.

On pig farms, animals are fed according to various feeding regimens. No effect on the efficacy of microbial phytase was shown, neither of feeding level (2.3 and 2.8 times maintenance requirement for metabolizable energy), nor of feeding frequency between two and seven times a day, while at feeding once a day the efficacy was slightly decreased (Mroz *et al.,* 1994a).

Effect of microbial phytase on digestibility/availability of other phytate-bound nutrients

Phytate complexes may be formed with various di- and trivalent cations, as well as with protein (Wise, 1980). In this paragraph the effect of microbial phytase on the release of complexed minerals and organic components are listed. To quantify the effect on increase of Ca digestibility, we calculated the ratio between the increase in amount of digestible Ca to the amount of digestible P.

The ratios in our own experiments (n=12) were on average 0.55±0.19, while those from data in the literature (n=20) were significantly higher 0.84±0.33. We have no clear explanation for this difference, but the levels of dietary Ca and digestible P play certainly a part (Mroz *et al.,* 1994b). By using 500 FTU/kg of feed, generating 0.8 g digestible P/kg and therefore between 0.4 and 0.7 g digestible $Ca.kg^{-1}$ is generated. Assuming a Ca digestibility of supplemental limestone of 60%, then between 1.7 and 3.0 g less $limestone.kg^{-1}$ of feed may be supplied. Microbial phytase also exerted a positive effect on Ca retention in broilers (Schöner *et al.,* 1991,1993,1994; Kornegay, 1995); 500 FTU was equivalent to 0.6 to 1.1 g of available Ca in these studies.

Microbial phytase can also improve the apparent absorption of Mg, Zn, Cu and Fe. The results listed in Table 3 show that the apparent digestibility of Mg, Zn, Cu and Fe increased in most studies with pigs. The effects, however, largely depend on the concentrations of the minerals in the basal diets. Plasma level of Zn is a good parameter for the Zn status of the animal. Higher Zn levels in plasma could be demonstrated by supplementary microbial phytase (Lei *et al.,* 1993b; 1993c; Pallauf *et al.,* 1992b; 1994b). Jongbloed *et al.* (1995c) showed that plasma Zn levels remained constant in a Zn-deficient diet supplied with microbial phytase, but plasma levels declined by 50% in the absence of this enzyme. In broilers, Thiel and Weigand (1992) and Yi (1995) confirmed the positive effect of microbial phytase on Zn availability. Roberson and Edwards (1992), however, did not observe this effect, probably because of high inclusion levels of Zn in their diets.

Table 3. Improvement of availability (% units) of some minerals by microbial phytase using the balance technique

FTU/kg	Mg	Zn	Cu	Fe	Reference
500	7	3	0	0	Näsi, 1990
1000	11	11	5	5	Pallauf *et al.,* 1992b
1200	6	3	-	-	Näsi and Helander, 1994
700	5	10	-	-	Pallauf *et al.,* 1994a
700	-1	-	-	-	Pallauf *et al.,* 1994b
500	2	-	-	-	Windisch *et al.,* 1994
1000	7	22	-	-	Lantzsch *et al.,* 1995
250	15	33	14	-	Lantzsch and Drochner, 1995

Guillot and Rambeck (1995) observed in rats, Japanese quails and turkeys and Rambeck *et al.* (1994) in broilers that microbial phytase reduced cadmium accumulation in liver and kidneys by 25 to 35%. This might be explained by the higher bioavailability of minerals and trace elements like calcium, zinc and iron, which are known to reduce the bioavailability of cadmium in different animal species.

Information concerning the effect of microbial phytase on protein digestibility is limited and inconsistent. Officer and Batterham (1992) reported that ileal digestibility of crude protein and essential amino acids in pigs increased by 7-12 percentage units. Mroz *et al.* (1994a) observed a lower effect, i.e., by 3.5 percentage units and in a second experiment Kemme *et al.* (1995a) also showed a similar increase in ileal digestibility for several amino acids (Table 4). Lantzsch *et al.* (1995), however, could not demonstrate an enhanced ileal digestibility or retention of N in piglets fed diets supplemented with 700 $FTU.kg^{-1}$ of feed. Yi (1995) showed in an experiment with turkey poults an increase of apparent ileal N digestibility from 0 to 2.7%, whereas digestibility of the essential amino acids was increased by 0 to 3.2%.

We also calculated the effect of microbial phytase on apparent faecal digestibility of some proximate nutrients in pigs. In our studies microbial phytase enhanced average dry matter digestibility by 0.54±0.81 percentage units (n=18), organic matter by 0.33±0.72 (n=9) and N by 1.21±1.73 (n=7). From these data it can be concluded that there is a tendency for increased faecal digestibilities of dry matter, organic matter and N by using supplementary microbial phytase. Data from literature showed a slightly different picture. The faecal digestibilities of dry matter and organic matter were slightly reduced by 0.11±0.69 (n=9) and 0.37±0.57 (n=3), respectively, while digestibility of N was increased by 0.60±1.66 (n=10) by supplementary phytase.

Table 4. Effect of microbial phytase (800 to 900 $FTU.kg^{-1}$) on apparent ileal digestibility of N and some amino acids

Nutrient	Phytase			Phytase		
	no[†]	yes[†]	SSD[†]	no[††]	yes[††]	SSD[††]
N	71.7	74.2	NS	74.2	75.8	NS
Lysine	81.0	81.9	NS	77.5	79.9	*
Methionine	76.9	80.6	**	80.6	81.7	NS
Cysteine	70.5	74.1	NS	73.5	72.2	NS
Tryptophan	72.4	73.7	NS	68.3	72.7	*
Isoleucine	80.1	79.8	NS	77.9	80.0	*
Threonine	73.8	72.0	NS	68.3	71.2	**

[†]Mroz *et al.,* 1994a
[††]Kemme *et al.,* 1995a)
NS: non significant; *P<0.05; **P<0.01

The effect of microbial phytase on performance

For a proper judgement of the effect of microbial phytase on performance of pigs both a negative control and a positive control treatment are required. In most cases a negative control is present as a basal diet mostly without supplementary feed phosphate and microbial phytase. As positive control we chose 11 experiments in which an addition of 1 gram P or more from a feed phosphate was used (Beers and Jongbloed, 1992a; Beers and Jongbloed, 1992b; Cromwell *et al.,* 1993; Hoppe *et al.,* 1993; Ketaren *et al.*, 1993; Windisch *et al.,* 1994; Borggreve, 1995; Campbell *et al.,* 1995; Helander, 1995; Helander and Partanen, 1995; Yi, 1995). The relative values were calculated whereby those of the negative control diet were set on 100. The results are presented in Table 5.

Data in Table 5 show that the performance of both the positive control and the phytase groups were superior to the negative control group. The positive control group and the phytase supplemented group were almost identical. Surprisingly, performance of the pigs on the positive control group with supplementary phytase was slightly better than those without phytase. This may imply that either the P requirement was not yet met or there is another positive effect of phytase on performance. No differences in slaughter quality could be noticed between the positive control group and the phytase group (n=2). In experiments with poultry (e.g. Simons and Versteegh, 1993; Yi, 1995) performance was improved in a similar way as with pigs.

Table 5. Relative performance of pigs using the negative control diet as reference (100)

	Negative control (n=11)	Positive control (n=11)	Negative+ phytase (n=11)	Positive+ phytase (n=6)
Growth rate	100	115.0±6.5	116.7±10.6	121.9±5.5
Feed intake	100	105.4±5.2	107.6± 7.8	108.6±3.2
Feed conv. ratio	100	93.0±4.9	93.2± 5.0	89.0±4.9

+Environmental impact of microbial phytase

In the Netherlands in a monitoring programme the concentrations of several minerals are occasionally monitored. Excretion of P by several categories of pigs and poultry can therefore be estimated. In Table 6 a survey is presented for growing-finishing pigs from 1973 to 1994. In that period performance increased considerably. Growth rate increased in that period from 625 to 730 $g.day^{-1}$, while feed conversion ratio improved from 3.37 to 2.80. Despite the better performance P content in pig diets decreased more than 2.0 $g.kg^{-1}$. From 1988 onwards, it is common practice to feed a starter diet and a grower-finisher diet. The excretion of P in growing-finishing pigs has more than halved in 20 years. This lower P excretion has, apart from increased nutritional knowledge, undoubtedly been stimulated by legislation based on P. Microbial phytase is commercially available in the Netherlands since 1992 and is used in 70% of the pig feeds now. The incorporation levels of microbial phytase will increase in the coming years resulting in a further decrease of P excretion by pigs and poultry.

Table 6. Mean excretion of P of a growing-finishing pig from 25 to 110 kg in the Netherlands

Year	P feed ($g.kg^{-1}$)	Feed conv.	Excretion ($kg.pig^{-1}$)
1973	7.4	3.37	1.62
1983	6.2	3.08	1.18
1988	6.0/5.0	2.96	0.88
1992	5.5/4.9	2.87	0.80
1994	5.5/4.8	2.80	0.75

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