

EFFECT OF PHYTASE SUPPLEMENTATION ON GROWING PIGS PERFORMANCE

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Dietary concentrations of phytate are crucial for its anti-nutritive properties and its negative impact on P availability. The increase of dietary phytate level is shown to increase endogenous losses of amino acids and minerals in pigs. The partial availability of the P component of phytate to simple-stomached species attains importance as the world's rock phosphate reserves are not renewable, which could lead to a P supply crisis in the future. Supplementing phytase is becoming increasingly common as a method to improve the availability of P in plant ingredients containing high levels of phytate P.

*Forty-eight pigs (Swedish Landrace boars × Dutch landrace sows) weaned at day 35 with an initial BW of 8.72 ± 0.28 kg were used for a 40-day weaner performance study. The study was structured as a complete randomized design to evaluate the response of weaner pigs to four concentrations of microbial phytase produced by *Aspergillus niger*: (T1) basal diet; (T2) basal diet + 1000 FTU/kg; (T3) diet with decreased dicalcium phosphate + 1000 FTU/kg; and (T4) diet with no dicalcium phosphate + 1000 FTU/kg.*

Control group of piglets (T1) has achieved standard body mass while addition of phytase in meal increased body mass by 6.59% in T2 and 7.52% in T3. Phytase supplementation prevented decreased body weight gain diets where available phosphorous level was reduced by 50. The amount of consumed feed per day was not significantly different. Feed efficiency of T2 and T3 groups was by 3.23% better and of T4 for 11.29% lower compared to the control group of piglets (T1).

Lower production results achieved by the use of low phosphorous diets can be avoided to a certain level by the use of microbial phytase. The use of phytase in pig diet significantly improved phosphorous availability, as well as of other mineral substances from the phytate complex.

Key words: growth performance, phytase, pigs, supplementation

INTRODUCTION

Phosphorous, necessary for skeleton formation and growth, is involved in the metabolism of lipids, carbohydrates and fats. It is a constitutive element of energy rich molecules (adenosine triphosphate and creatine phosphate), nucleic acids and of blood and rumen buffers. In cases of low phosphorous deficiency the first clinical symptom is decreased appetite followed by lower availability of nutrient matters, decreased body mass and production results. Longer deficiency leads to inadequate bone mineralization which results in soft fragile bones in young (*rachitis*) and old animals (*osteomalacia*) (Šefer and Sinovec, 2008).

Phytate was first identified more than a century ago (Hartig, 1855). Phytate and phytate-bound phosphorus (P) is present in all diets and the partial availability of phytate-P has long been recognized (Lowe *et al.*, 1939). The partial availability of the P component (282 g kg^{-1}) of phytate to simple-stomached species assumes importance as the world's rock phosphate reserves are not renewable, which could lead to a P supply crisis in the future (Mullaney *et al.*, 2000). Environmental regulations have set strict limits on the level of P to be discharged in swine effluents (Vats *et al.*, 2005; Selle and Ravindran, 2008). In addition, the inclusion of excessive levels of inorganic P results in higher feed costs. The global harvest of crop seeds and fruits contains an estimated 14.4 million tonnes of phytate-P, which is equivalent to 65% of annual sales of P as fertilizers (Lott *et al.*, 2000). Logically, dietary concentrations of phytate are crucial to its anti-nutritive properties and its negative impact on P availability. The addition of sodium phytate to glucose-based diets has been shown to increase endogenous losses of amino acids and minerals (Cowieson *et al.*, 2004). It follows that responses to phytase will be more pronounced with increasing dietary phytate levels and indications of this have been recorded in poultry (Ravindran *et al.*, 2006) and pigs (Selle *et al.*, 2003). Because available P reserves are finite, even higher costs are expected in the future (Cordell *et al.*, 2009). Hence, there is a need to develop nutritional strategies which use the concept of P bioavailability, thus, we can provide adequate P to satisfy the animal's needs.

Supplementing phytase is becoming increasingly common as a method to improve the availability of P in plant ingredients containing high levels of phytate P (Selle and Ravindran, 2008). Phytase feed enzymes have a more general application as their substrate is invariably present in pig and poultry diets and their dietary inclusion economically generates bioavailable P and reduces the P load on the environment.

Animal nutritionists have long regarded phytate as both indigestible and an anti-nutritional factor for non-ruminant animals (Swick and Ivey, 1992). Phytate is ubiquitous in plant-sourced feed ingredients as it serves as a P reservoir during seed germination.

The inclusion of feed enzymes in poultry diets to enhance nutrient utilization and performance by counteracting the negative influence of targeted substrates has become common place within the last two decades (Selle and Ravindran, 2007).

Although phytase activity was first detected in rice bran more than a century ago (Suzuki *et al.*, 1907), attempts to develop a phytase feed enzyme did not commence until 1962 in North America (Wodzinski and Ullah, 1996). Nevertheless, it was not until 1991 that the first phytase feed enzyme became commercially available, which was largely in response to the legislation designed to limit P pollution of the environment in the Netherlands. Phytase, which occurs widely throughout nature, is the enzyme needed to degrade phytate and it has the capacity to hydrolyze phytate and release inorganic P (Selle and Ravidran, 2007).

Several distinct microbial phytase products are now commercially available. The three commonly used phytase feed enzymes are derived from *A. niger*, which is a 3-phytase (EC 3.1.3.8) and *Peniophora lycii* and *Escherichia coli*, which are 6-phytases (EC 3.1.3.26). Phytase feed enzymes may be included in poultry rations as granulates or as liquids, via post-pelleting application systems, to avoid thermostability problems at high pelleting temperatures (>80°C). There are, however, perceived advantages in inherently heat stable phytase feed enzymes that can withstand steam-pelleting, as illustrated by the investigations of Wyss *et al.* (1998) and Garrett *et al.* (2004).

In the *A. niger* phytase introduced in 1991, phytase activity is defined as phytase units (FTU), where one FTU is the amount of enzyme that liberates 1 μmol inorganic orthophosphate/min from 0.0051 mol L⁻¹ sodium phytate at pH 5.5 and at a temperature of 37°C (Engelen *et al.*, 1994).

The hypothesis of this study was that inclusion of phytase (1000 phytase units (FTU)/kg) in a low-P weaned pig diets will affect available P (aP) and prevent reduced growth performance when fed low-P weaned pig diets.

MATERIAL AND METHODS

Animals and procedures. Forty-eight pigs (Swedish Landrace boars × Dutch Landrace sows) weaned at day 35 with an initial BW of 8.72±0.28 kg were used for a 40-day weaner performance study. The pigs were penned (1.68×1.22 m) in a house with fully slatted floors, and each pen contained one male and one female, resulting in 12 replicates per treatment. Feed and water via nipple drinkers were available *ad libitum*. Room temperature was set at 26°C for the first week and was reduced by 2°C per week to 22°C. Animals were weighed on days 0, 20 and 40. Feed samples were collected each day and retained for chemical analysis at -20°C. Samples of freshly voided feces were collected on days 0, 20 and 40 and frozen (-20°C) for laboratory analysis (determination of available P (aP) content).

Experimental design and diets. The study was designed as a complete randomized design to evaluate the response of weaner pigs to four concentrations of phytase: (T1) basal diet; (T2) basal diet + 1000 FTU/kg; (T3) diet with decreased dicalcium phosphate + 1000 FTU/kg; and (T4) diet with no dicalcium phosphate + 1000 FTU/kg. The microbial phytase used was produced by *Aspergillus niger* (Natuphos® BASF Corporation, Ludwigshafen, Germany).

Phytase was initially mixed with approximately 10 kg of ground wheat prior to being added to the mixer to ensure homogeneity. Each diet was manufactured

in an 800 kg batch. All diets were formulated to have identical concentrations of Ca, digestible energy and ileal digestible lysine (INRA-AFZ, 2004). All other amino acid requirements were provided relative to lysine according to the ideal protein concept (NRC, 1998). All diets were fed as meals.

The starter diets (up to 15 kg BW) for T1, T2 and T3 groups contained 8.0, 8.0 and 6.2 g/kg of total P (tP) respectively, which was in accordance to the recommendations of Serbian Rule Book (2010) and NRC (1998) standards for weaner pigs. The diet for group T4 contained 4.4 g/kg of total P (tP) which was below both mentioned standards. The diets phytic P (pP) content was identical in all treatment groups (2.3 g/kg). Composition and chemical analysis of starter diets (up to 15 kg BW) are presented in Table 1.

Table 1. Composition and chemical analysis of the starter diets (up to 15 kg BW)

| Item | T1 | T2 ^a | T3 ^a | T4 ^a |
|---|-------|-----------------|-----------------|-----------------|
| Composition (% as-fed basis) | | | | |
| Corn | 65.5 | 65.5 | 65.7 | 65.8 |
| Soybean meal | 21.0 | 21.0 | 21.0 | 21.0 |
| Sunflower meal | 4.0 | 4.0 | 4.0 | 4.0 |
| Livestock yeast | 4.0 | 4.0 | 4.0 | 4.0 |
| Animal fat | 2.0 | 2.0 | 2.0 | 2.0 |
| Limestone flour | 1.1 | 1.1 | 1.5 | 1.9 |
| Dicalcium phosphate | 1.0 | 1.0 | 0.5 | – |
| Salt | 0.3 | 0.3 | 0.3 | 0.3 |
| Chromium (III) oxide (Cr ₂ O ₃) ^b | 0.1 | 0.1 | 0.1 | 0.1 |
| Minerals and vitamins ^c | 1.0 | 1.0 | 1.0 | 1.0 |
| Analyzed composition ^d (% DM) | | | | |
| Crude protein | 20.00 | 20.00 | 20.02 | 20.05 |
| Fat | 4.87 | 4.87 | 4.89 | 4.89 |
| Cellulose | 3.64 | 3.64 | 3.65 | 3.65 |
| Ca | 1.06 | 1.06 | 1.04 | 1.03 |
| tP (total P) | 0.80 | 0.80 | 0.62 | 0.44 |
| aP (available P) | 0.36 | 0.36 | 0.24 | 0.11 |
| Calculated composition ^e | | | | |
| Lysine | 1.08 | 1.08 | 1.08 | 1.08 |
| Methionine and cysteine | 0.61 | 0.61 | 0.61 | 0.61 |
| Tryptophan | 0.28 | 0.28 | 0.28 | 0.28 |
| Gross energy (MJ/kg) | 14.15 | 14.15 | 14.19 | 14.23 |
| pP (phytic P) | 0.23 | 0.23 | 0.23 | 0.23 |

^aExperimental diets were supplemented with phytase (1000 FTU/kg for T2, T3 and T4 diets). Phytase (Natuphos[®] BASF Corporation, Ludwigshafen, Germany); ^bChromium(III) oxide (Cr₂O₃) – as

digestibility indicator; ^cPremix provided per kg of complete diet: 3 mg retinol, 0.05 mg cholecalciferol, 40 mg α -tocopherol, 90 mg copper as copper sulphate, 100 mg iron as iron sulphate, 100 mg zinc as zinc oxide, 0.3 mg selenium as sodium selenite, 25 mg manganese as manganous oxide and 0.2 mg iodine as calcium iodate on a calcium sulphate/calcium carbonate carrier; ^dChemically determined (% of Dry Matter – %DM) (n=12); ^eINRA-AFZ (2004).

The grower diets (from 15 to 25 kg BW) for groups T1 and T2 contained 6.1 g/kg of total P (tP) which was in accordance to the recommendations of both Serbian Rule Book (2010) and NRC (1998) standards for weaner pigs. The grower diets of T3 and T4 groups contained 5.3 and 4.5 g/kg of total P (tP), respectively which was below the mentioned recommendations. The diets phytic P (pP) content was identical in all treatment groups (2.4 g/kg). Composition and chemical analysis of grower diets (from 15 to 25 kg BW) are presented in Table 2.

Table 2. Composition and chemical analysis of the grower diets (from 15 to 25 kg BW)

| Item | T1 | T2 ^a | T3 ^a | T4 ^a |
|--|-------|-----------------|-----------------|-----------------|
| Composition (% as-fed basis) | | | | |
| Corn | 59.6 | 59.6 | 59.9 | 60.2 |
| Soybean meal | 23.0 | 23.0 | 23.0 | 23.0 |
| Sunflower meal | 6.0 | 6.0 | 6.0 | 6.0 |
| Livestock yeast | 5.0 | 5.0 | 5.0 | 5.0 |
| Animal fat | 2.0 | 2.0 | 2.0 | 2.0 |
| Limestone flour | 1.0 | 1.0 | 1.7 | 2.4 |
| Dicalcium phosphate | 2.0 | 2.0 | 1.0 | – |
| Salt | 0.3 | 0.3 | 0.3 | 0.3 |
| Chromium(III) oxide (Cr ₂ O ₃) ^b | 0.1 | 0.1 | 0.1 | 0.1 |
| Minerals and vitamins ^c | 1.0 | 1.0 | 1.0 | 1.0 |
| Analyzed composition ^d (% DM) | | | | |
| Crude protein | 18.26 | 18.26 | 18.27 | 18.28 |
| Fat | 5.02 | 5.02 | 5.03 | 5.03 |
| Cellulose | 3.34 | 3.34 | 3.35 | 3.35 |
| Ca | 0.82 | 0.82 | 0.83 | 0.83 |
| tP (total P) | 0.61 | 0.61 | 0.53 | 0.45 |
| aP (available P) | 0.22 | 0.22 | 0.17 | 0.11 |
| Calculated composition ^e | | | | |
| Lysine | 0.96 | 0.96 | 0.96 | 0.96 |
| Methionine and cysteine | 0.55 | 0.55 | 0.55 | 0.55 |
| Tryptophan | 0.26 | 0.26 | 0.26 | 0.26 |
| Gross energy (MJ/kg) | 14.28 | 14.28 | 14.29 | 14.31 |
| pP (phytic P) | 0.24 | 0.24 | 0.24 | 0.24 |

^aExperimental diets were supplemented with phytase (1000 FTU/kg for T2, T3 and T4 diets). Phytase (Natuphos[®] BASF Corporation, Ludwigshafen, Germany); ^bChromium(III) oxide (Cr₂O₃) – as digestibility indicator; ^cPremix provided per kg of complete diet: 3 mg retinol, 0.05 mg cholecalciferol, 40 mg α -tocopherol, 90 mg copper as copper sulphate, 100 mg iron as iron sulphate, 100 mg zinc as zinc oxide, 0.3 mg selenium as sodium selenite, 25 mg manganese as manganous oxide and 0.2 mg iodine as calcium iodate on a calcium sulphate/calcium carbonate carrier; ^dChemically determined (% of Dry Matter – %DM) (n=12); ^eINRA-AFZ (2004)

Laboratory analysis of samples. Diets were analyzed for dry matter, fat, cellulose, proteins and crude ash (AOAC, 1997). The dry matter were determined after drying for 24 h at 100°C and the crude ash content was determined after ignition of a weighed sample in a muffle furnace at 550°C for 6 h. The ash was then digested in aqua regia (HCl/HNO₃ mixture). This solution was used for P and Ca determination. The Ca concentration was determined using an atomic absorption spectrophotometer (Perkin-Elmer Analyst 700 MHS) using the method of Ramakrishna *et al.* (1968). The concentration of P was determined spectrophotometrically using the method of Cavell (1955). The dietary gross energy (MJ/kg), concentrations of lysine, tryptophan, methionine and cysteine, as well as pP (phytic P) were calculated according to INRA-AFZ (2004).

Calculations and statistics. Statistical analysis of intergroup differences of means was performed by ANOVA, Tukey multiple comparison test was used. Software package Prism Pad v. 5.0 (Graph Pad Software Inc., San Diego, CA, USA) was used for statistical evaluation. Data were expressed as means \pm standard deviation. Differences with $p < 0.05$ were considered statistically significant.

RESULTS

During the study piglets from all experimental groups were of good health, no deaths were recorded, nor loss of appetite. At the beginning of the study body mass of piglets was not significantly different between experimental groups ($p > 0.05$). Differences were not significant nor after the first and second phase of the study (day 20th and 40th), which is shown in Table 3.

Table 3. Piglet body mass (kg)

| Day of the study | T1 | T2 | T3 | T4 |
|------------------|------------------|------------------|------------------|------------------|
| 1 st | 8.71 \pm 0.90 | 8.71 \pm 1.00 | 8.73 \pm 0.88 | 8.73 \pm 1.17 |
| Index | 100.00 | 100.00 | 100.23 | 100.23 |
| 20 th | 15.35 \pm 1.81 | 15.45 \pm 1.94 | 15.13 \pm 2.39 | 14.51 \pm 2.59 |
| Index | 100.00 | 100.65 | 98.57 | 94.53 |
| 40 th | 23.67 \pm 3.56 | 25.23 \pm 2.51 | 25.45 \pm 3.36 | 22.98 \pm 2.56 |
| Index | 100.00 | 106.59 | 107.52 | 97.08 |

Average daily body weight gain (BWG), in the period from 1st to 20th day of the study, was significantly lower in the T4 group of piglets (fed diet without supplemented inorganic phosphorous plus added phytase enzyme) compared to the other groups of piglets ($p < 0.05$). In the period from the 21st to 40th day of the study, T2 and T3 group of piglets achieved better daily body weight gain compared to T1 and T4 groups of piglets ($p < 0.05$). The average daily body weight gain for the whole study period was lowest in group T4 and highest in group T3. The measured daily body weight gain of the piglets in T4 was significantly lower compared to T3 ($p < 0.01$) and T2 group ($p < 0.05$), the results are shown in Table 4.

Table 4. Average daily body weight gain (BWG) (kg)

| Day of the study | T1 | T2 | T3 | T4 |
|--------------------------------------|----------------------------|----------------------------|----------------------------|------------------------------|
| 1 st to 20 th | 0.348±0.051 ^a | 0.363±0.052 ^b | 0.367±0.047 ^c | 0.272±0.038 ^{a,b,c} |
| Index | 100.00 | 104.31 | 105.46 | 78.16 |
| 21 st to 40 th | 0.380±0.049 ^{a,b} | 0.452±0.025 ^{a,c} | 0.453±0.049 ^{b,d} | 0.384±0.027 ^{c,d} |
| Index | 100.00 | 118.95 | 119.21 | 101.05 |
| 1 st to 40 th | 0.364±0.033 | 0.406±0.053 ^a | 0.408±0.029 ^A | 0.345±0.021 ^{A,a} |
| Index | 100.00 | 112.78 | 113.33 | 95.83 |

Same letters differ at a, b, c, d $p < 0.05$, A $p < 0.01$.

The amount of consumed feed per day measured in this study is shown in Table 5. No significant differences in the amount of feed consumed between control and all experimental groups of piglets during all study period (1st till 40th day of the study) were observed ($p > 0.05$). However, it was observed that the control group of piglets (T1) consumed less feed (per day) compared to T2, T3 and T4 group of piglets (for 7.84, 8.73, and 5.77% respectively).

Table 5. Feed consumption per day (kg)

| Day of the study | T1 | T2 | T3 | T4 |
|--------------------------------------|--------|--------|--------|--------|
| 1 st to 20 th | 0.520 | 0.528 | 0.512 | 0.538 |
| Index | 100.00 | 100.57 | 97.52 | 102.48 |
| 21 st to 40 th | 0.827 | 0.930 | 0.958 | 0.892 |
| Index | 100.00 | 112.45 | 115.84 | 107.86 |
| 1 st to 40 th | 0.676 | 0.729 | 0.735 | 0.715 |
| Index | 100.00 | 107.84 | 108.73 | 105.77 |

Feed efficiency (feed conversion ratio FCR) is shown in Table 6. In the period from 1st to 20th day the lowest feed conversion ratio was measured in group T2 and highest in group T4. The situation in the second phase of the study (21st to 40th day) was similar, but the difference was more pronounced. Taking into

account the entire study period (1st to 40th day) FCR in T2 and T3 was by 3.23% lower and in T4 by 11.29% higher compared to the control group of piglets (T1).

Table 6. Feed efficiency (kg)

| Day of the study | T1 | T2 | T3 | T4 |
|--------------------------------------|--------|-------|--------|--------|
| 1 st to 20 th | 1.58 | 1.57 | 1.60 | 1.86 |
| Index | 100.00 | 99.30 | 101.20 | 117.70 |
| 21 st to 40 th | 2.18 | 2.06 | 2.11 | 2.32 |
| Index | 100.00 | 94.50 | 96.79 | 106.42 |
| 1 st to 40 th | 1.86 | 1.80 | 1.80 | 2.07 |
| Index | 100.00 | 96.77 | 96.77 | 111.29 |

DISCUSSION

The phosphorous deficiency in nutrition due to its role in the processes of energy transfer disturbs biosynthetic reactions and leads to depressed growth of the young, as well as lower production results of mature animals (Šefer, 2002). In our study the control group of piglets (T1) achieved standard body mass for its breed and age while addition of phytase in the meal increased body mass by 6.59% and 7.52% in T2 and T3, respectively. On the contrary in group T4 (no inorganic phosphorous supplemented plus phytase) the decrease in body mass by 2.92% compared to the control group (T1) of piglets was not statistically significant ($p > 0.05$). Judging by the results in group T2 and T3, phytase addition completely prevented negative effects of lower total available phosphorous. The finding that phytase had a weak effect on group T4 is in accordance to the findings of Qian *et al.* (1996). The mentioned authors concluded that phytase is most efficient in the diets with low available phosphorous and in diets where Ca to P ratio is 2:1 and that the positive effects of phytase supplementation decrease together with the decrease of phosphorous content in the diets. A more controversial area is whether or not phytase enhances growth performance of pigs offered P adequate diets and, if so, the genesis of these responses should be elucidated. Beers and Jongbloed (1992) reported the first example of phytase enhancing performance was in pigs offered P-adequate diets. They reported that 1450 FTU kg^{-1} phytase increased growth rate by 12.8%, feed intake by 8.5% and feed efficiency by 4.4% in diets for weaner pigs containing to 2.9 g kg^{-1} nonphytate P. As these responses could not be attributed to enhanced P digestibility, one explanation was that phytase may have increased protein/amino acids digestibility, as it may be relevant that the diets contained relatively low protein (185 g kg^{-1}) levels.

Phytate is a polyanionic molecule with the potential to chelate positively charged nutrients, which is almost certainly fundamental to the anti-nutritive properties of phytate. These anti-nutritive properties require further investigation,

but phytate probably compromises the utilization of protein/amino acids, energy, calcium and trace minerals (Selle and Ravidran, 2007).

Average daily body weight gain is considered a reliable indicator of feed quality, especially in the investigation of phosphorous bioavailability in feedstuffs (Sefer, 2002).

Average daily body weight gain (BWG), in the period from the 1st to the 20th day of the study, was significantly lower in the T4 group of piglets (fed the diet without supplemented inorganic phosphorous plus added phytase enzyme) compared to the other groups of piglets ($p < 0.05$). Phytase supplementation in groups T2 and T3 prevented decreased body weight gain when compared to the control, however this was not the case for group T4, where available phosphorous was at only 30% of the level of available phosphorous in the control group (T1). In the period from the 21st to the 40th day of the study, T2 and T3 groups of piglets had achieved better daily body weight gain compared to T1 and T4 groups of piglets ($p < 0.05$). The average daily body weight gain for the whole study period was lowest in T4 and highest in T3 group of piglets. The fact that the measured daily body weight gain of the piglets in T4 was significantly lower compared to T3 ($p < 0.01$) and T2 group ($p < 0.05$) is worth mentioning.

Previous research has demonstrated that phytase supplementation in weaner pig diets has the ability to improve weaner pig feed efficiency (Brana *et al.*, 2006; Woyengo *et al.*, 2008). Exogenous phytase has been shown to increase blood glucose concentrations in pigs (Johnston *et al.*, 2004; Kies *et al.*, 2005) and it is likely that phytate impedes glucose uptake in humans (Selle and Ravidran, 2007). Predictably, phytase feed enzymes have the capacity to enhance growth performance of pigs offered P-deficient diets (Selle and Ravidran, 2007). Similar 'extra-phosphoric' responses in growth performance of weaner pigs offered wheat-based diets were reported by Campbell *et al.* (1995). In this study, 500 FTU kg^{-1} phytase improved weight gain and feed efficiency of weaner pigs offered diets containing either 3.5 or 4.5 g kg^{-1} available P with an average of 14.4 and 9.2%, respectively. In contrast, however, Barnett *et al.* (1993) did not observe enhanced growth performance in weaner pigs offered conventional, wheat-based diets following 1000 FTU kg^{-1} phytase addition. However, retrospective estimates of dietary phytate concentrations indicated that there were substantial differences in substrate levels between the two studies (Selle *et al.*, 1997). In the Barnett *et al.* (1993) study the diets contained approximately 2.0 g kg^{-1} phytate-P; whereas, in the Campbell *et al.* (1995) study, the diets contained an estimated 3.9 g kg^{-1} phytate-P. These contrasting results suggest that dietary substrate levels are an important determinant of the magnitude of phytase responses.

The amount of consumed feed per day measured in our study was not significantly different ($p > 0.05$). However, it was observed that the control group of piglets (T1) consumed less feed daily, compared to groups T2, T3 and T4. This finding is not surprising since the fact that dietary phosphorus deficiency has an immediate depressing effect on appetite, growth rate, and feed efficiency of swine (NRC, 1998; Crenshaw, 2001). Our finding is similar to the findings of Harper *et al.* (1997) who have found that supplemental phytase can prevent decreased

appetite in low phosphorous diets and even increase consumption of diets low in available phosphorous.

Feed efficiency (feed conversion ratio) as an interaction between body weight gain and amount of feed consumed was different between groups. Feed efficiency for T2 and T3 groups was by 3.23% better and for T4 by 11.29% lower compared to the control group of piglets (T1) throughout the study period. The attitude towards phytase effects on feed efficiency is somewhat controversial. Cromwell *et al.* (1993) found that phytase supplementation of diets low in phosphorous affects body weight gain and feed consumption without effects on feed efficiency. On the contrary Yi *et al.* (1996) and Harper *et al.* (1997) reported that besides other positive effects phytase supplementation increased feed efficiency.

In a series of three feeding studies reported by Selle *et al.* (2003), microbial phytase (550 to 750 FTU kg⁻¹) generated overall improvements in weight gain by an average of 9.4% (401 versus 367 g day⁻¹) and feed efficiency by 6.1% (1.23 versus 1.31) in weaner pigs offered phosphorus-adequate, lysine-deficient, wheat based diets. Also, there was a positive correlation between percentage improvements in feed efficiency in response to phytase and dietary phytate contents ($r=0.923$; $P=0.005$). In one experiment, diets containing 1.2, 2.2 and 3.2 g kg⁻¹ phytate-P were offered to pigs without and with 625 FTU kg⁻¹ phytase. While responses to phytase appeared to be more pronounced in the high phytate diets (weight gain: 12.1%; feed efficiency: 10.8%) than in the low phytate diets (weight gain: -1.30%; feed efficiency: 0.0%), treatment interactions were not statistically significant.

The validity of responses to phytase in P adequate diets have been challenged on the basis that enzymic 'side-activities' in phytase preparations may be generating independent, positive effects (Farrell and Martin, 1998; Driver *et al.*, 2006). While side-activities have been identified in phytase feed enzymes (Zyla *et al.*, 2000) it is questionable if they could exert any tangible, independent effects at recommended inclusion rates although it is possible that they may facilitate phytase activity. It is understood that certain phytase feed enzymes contain acid phosphatase side-activity, which would augment phytate hydrolysis by phytase (Zyla, 1993).

Finally, by observing all production results obtained in this study we can confirm that the best production results were achieved in the group of pigs fed a diet with the recommended amount of phosphorous plus supplemental phytase. Interestingly, feeding diets with a lower amount of mineral source of phosphorous supplemented with phytase resulted in nearly as good production results as with adequate phosphorous diet thus confirming the fact that phytase has successfully freed the necessary amount of phosphorous from its phytic form.

In summary, based on the results of our study we can conclude that lower production results achieved by the use of diets low in phosphorous can be avoided to a certain level by the use of microbial phytase. The use of phytase in pig diet significantly improved phosphorous availability, as well as of other mineral matters from the phytate complex and therefore reduces soil contamination by undigested nutrient matters. Finally, we can conclude that up to 30% of total or up

to 50% of available phosphorous can be efficiently substituted by the inclusion of 1000 FTU/kg of phytase.

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UTICAJ DODAVANJA FITAZE U HRANU NA ZDRAVSTVENI STATUS I PROIZVODNE REZULTATE SVINJA U TOVU

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SADRŽAJ

Zbog izraženih antinutritivnih svojstava fitata i negativnog uticaja na iskorišćavanje fosfora, njihova koncentracija u hrani je od velikog značaja. Poznato je da visoka koncentracija fitata u hrani povećava endogene gubitke amino kiselina i minerala kod svinja. Delimična dostupnost fosfora iz fitata kod monogastičnih životinja još više dobija na značaju usled činjenice da svetske rezerve mineralnog fosfora iz stena nisu obnovljive i da to može dovesti do krize u snabdevanju fosforom u budućnosti. Dodavanje fitaze postaje sve popularnije kao metod za povećanje dostupnosti fosfora u biljnim sirovinama koje sadrže visoku koncentraciju fosfora. Četrdeset osam prasadi (Švedski Landras nerast × Danski Landras krmača) zalučenih u starosti od 35 dana prosečne telesne mase $8,72 \pm 0,28$ kg je uključeno u 40-to dnevni ogled ispitivanja proizvodnih rezultata. Ogled je dizajniran sa ciljem da se ispita uticaj dodatka tri koncentracije fitaze poreklom od *Aspergillus niger* i to: (T1) bazalni obrok; (T2) bazalni obrok + 1000 FTU/kg; (T3) obrok sa smanjenom koncentracijom dikalcijum fosfata + 1000 FTU/kg; and (T4) obrok bez dodatog dikalcijum fosfata + 1000 FTU/kg.

Kontrolna grupa prasadi (T1) je postigla uobičajenu telesnu masu, dok je dodatak fitaze u obrok povećavao telesnu masu za 6,59% u T2 i 7,52% u T3. Dodavanje fitaze je preveniralo smanjenje prirasta kod smeša gde je koncentracija fosfora bila niža za 50% od potreba. Količina konzumirane hrane se nije značajnije razlikovala među oglednim grupama. Konverzija hrane u grupama T2 i T3 je bila bolja za 3,23%, dok je u grupi T4 bila za 11,29% lošija u poređenju sa kontrolnom grupom (T1).

Lošiji proizvodni rezultati pri korišćenju obroka sa niskim sadržajem fosfora se mogu izbeći upotrebom fitaze mikrobijalnog porekla. Korišćenje fiteze u hrani za svinje u tovu značajno poboljšava iskoristljivost fosfora i drugih mineralnih materija poreklom iz fitatnog kompleksa.

