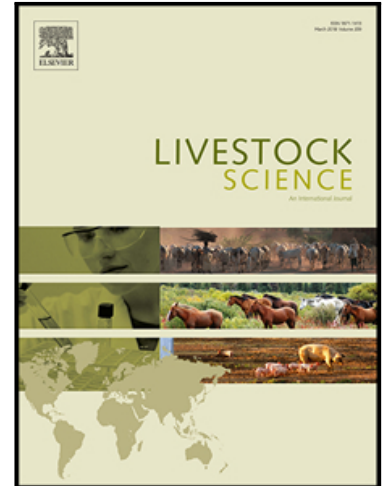


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Running Head: Carbohydrolase phytase effect on performance

Effect of a Combination Phytase and Carbohydrolase Enzyme Supplement on Growth Performance and Bone Mineralization of Pigs from Six Weeks to Slaughter at 105 kg.

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ABSTRACT: An experiment was conducted to assess the effect of a combination of carbohydrase (from *Talaromyces Versatilis*) and 6-phytase (from *Schizosaccharomyces Pombe*) multi enzyme complex (**mec**; Rovabio Max®, Adisseo, France) on the growth and bone mineralization of pigs fed maize-wheat-soybean meal diets. Pigs (n = 384) were selected at 28 days of age, penned in same gender pairs and fed a common acclimatization diet meeting animal requirements for 14 days. Four experimental diets were formulated for each of 4 growth stages from 42 days of age to slaughter at 147 days: 1) Positive control (**PC**), formulated to meet nutritional requirements; 2) Negative control 1 (**NC1**; DE x 0.985, CP x 0.985, -1.0 g Ca /kg and -1.2 g dig P /kg), 3) Negative control 2 (**NC2**; DE x 0.975, CP x 0.975, -1.0 g Ca /kg and -1.2 g dig P /kg) and 4) Negative control 3 (**NC3**; DE x 0.975, CP x 0.975, -1.5 g Ca /kg and -1.7 g dig P /kg). Negative control diets were also supplemented with mec resulting in 7 experimental treatments. Feed disappearance, wastage and individual pig live weight (**LW**) were recorded at the beginning and end of each growth phase. Reducing in dietary constituents (CP, DE, P and Ca) compared to PC reduced LW ($P < 0.001$), average daily feed intake (**ADFI**; $P < 0.01$), and average daily gain (**ADG**; $P < 0.001$) throughout the trial. Addition of mec to NC diets increased LW ($P < 0.001$), ADFI ($P < 0.001$) and ADG ($P < 0.001$) up to slaughter and improved feed conversion ratio (**FCR**; $P < 0.001$) to day 112 of the trial. There were increases in area bone mineral density (**aBMD**) of the foot from day 77 onwards ($P < 0.01$) and metacarpal aBMD ($P < 0.01$) from day 112 onwards when mec was added to NC diets although no effect ($P > 0.05$) on metacarpal Ca or P percentages was found. It was concluded that supplementing carbohydrase and phytase to low nutrient density diets can return the growth and FCR of the pigs as well as metacarpal and foot aBMD to the levels reached by pigs fed diets meeting nutrient recommendations.

Keyword: pig, performance, phosphorus, phytase, carbohydrase

INTRODUCTION

Wheat and maize are common ingredients in pig diets (Sauber and Owens 2001; Kim *et al.*, 2005). The presence of phytic acid (**PA**) and non-starch polysaccharides (**NSP**) in these ingredients can have anti-nutritional effects in monogastric animals, as they lack effective endogenous enzymes to digest these dietary components (Shaw *et al.*, 2006). Soybean and maize PA represent 1.4% and 0.9% of the dry matter; respectively, but it binds more than 60% of the total phosphorus (**P**) thereby rendering it unavailable for digestion (Shaw *et al.*, 2006). This often results in the total dietary P exceeding recommended levels for pigs' minimum requirements (Brana *et al.*, 2006; Létourneau-Montminy *et al.*, 2012). This excess can lead to environmental pollution and increased feed costs for the producer (Carpenter *et al.*, 2004; Johnston *et al.*, 2004; Brana *et al.*, 2006). In addition to PA, feedstuffs also contain NSP in the cell wall which reduces nutrient digestibility as a result of nutrient encapsulation and increasing digesta viscosity (Kim *et al.*, 2005). Together both PA and NSP reduce the nutrient availability in pig diets. This has encouraged the development and production of several commercial multi enzyme complex (**mec**) containing phytase and/or carbohydrase for inclusion in pig feeds in order to increase the availability of otherwise under-utilized nutrients (Kim *et al.*, 2005; Brana *et al.*, 2006; Woyengo *et al.*, 2008). The hypothesis of this study was that the inclusion of a mec containing carbohydrase (with a large range of enzyme activities) and phytase in reduced-nutrient (i.e. lower in energy, crude protein, calcium and phosphorus contents) pig diets will improve bone mineralization in pigs from 14 days post weaning until slaughter at 147 days of age while maintaining post-weaning pig growth and FCR.

MATERIALS AND METHODS

This experiment complied with EU Council Directive 91/630/EEC, which sets minimum standards for the protection of pigs, and EU Council Directive 98/58/EC, which concerns the protection of animals kept for farming purposes.

Diet

Dietary treatments are presented in Table 1. They consisted of 4 phases and were designed as follows: 1. positive control (**PC**) formulated to meet or exceed nutrient requirement of swine, according to National Research Council (NRC, 1998), for each of the growth phases; 2. Three negative control diets (**NC**) reduced in net energy (**NE**), crude protein (**CP**), digestible phosphorus (**dig P**) and calcium (**Ca**) content. NE and CP were 0.98 relative to PC for NC1 and 0.96 for NC2 and NC3. Mineral content of NC1 and NC2 was reduced by 1.0 g/kg and 1.2 g/kg for Ca and dig P. Mineral content of NC3 was reduced by 1.5 g/kg and 1.7 g/kg for Ca and dig P. The three NC diets were supplemented or not with exogenous enzyme and resulted in 3 supplementary dietary treatment NC1+; NC2+ and NC3+.

Enzyme preparation

A multi-enzyme complex (**MEC**) in liquid form was tested (Rovabio Max®, Adisseo France SAS, Antony, France), containing carbohydrases produced from the fermentation of *Talaromyces versatilis* and bacterial 6-phytase (EC 3.1.3.2.6) derived from *Escherichia coli* and expressed in *Schizosaccharomyces pombe*. The multi enzyme complex was applied after pelleting at a dose rate of 220 g/t of feed to provide a minimum of 1,100 visco-units of endo- β -1,4-xylanase, 100 azo- β -glucanase units of endo-1,3(4)- β -glucanase, and 500 phytase units (FTU)/kg of feed. One visco-unit of endo-1,4- β -xylanase activity is defined as the amount of mec reducing the viscosity of the solution, to give a change in relative fluidity of 1 dimensionless unit per minute per milliliter (or per gram) under the conditions of the assay

(pH 5.5 and 30°C). One azo- β -glucanase unit of endo-1,3(4)- β -glucanase activity is defined as the amount of mec releasing oligomers, which are soluble in ethanol, to give an absorbance of 0.820 units at 590 nm under the conditions of the assay (20 min at pH 4.6 and 30°C). One FTU is defined as the amount of mec that liberates one micromole of inorganic orthophosphate from phytic acid per minute at pH 5.5 and 37°C (Engelen et al., 1994).

Animals

The pigs used were the progeny of F1 (Landrace x Large White; Hermitage AI, Sion Rd., Kilkenny, Ireland) sows mated to HYLEAN MAXGROW (Hermitage AI) sires. Pigs 8.3 \pm 1.2 kg body weight (BW) were weaned at 28 days old and tagged for individual identification. Same sex pairs (n = 192) of similar weight were formed and penned together until slaughter. Pen pairs were blocked on weaning weight and sex, then randomly assigned and fed a sequence of diets to slaughter at 147 days (27 or 28 pens / Treatment). At Weaner stage (day 0 to day 35 post-weaning) pairs of pigs were housed in fully slatted pens (1.2 m x 0.9 m). Each pen had a single 300 mm wide stainless steel feeder (O'Donovan Engineering, Coachford, Co. Cork, Ireland). Water was available *ad libitum* from bowls (BALP, Charleville-Mezieres, Cedex, France). Temperature was maintained at 28-30 °C in the first week after weaning and reduced by 2 °C per week to 22 °C. At grower-finisher stage (day 35 post-weaning to 147 days of age), pairs of pigs were penned in fully slatted pens (1.81m x 1.18m) with steel rail partitions. Air temperature was maintained at 20 to 22 °C. The feeders were stainless steel dry feed hoppers 30 cm in width (O'Donovan Engineering, Coachford, Co. Cork, Ireland). There was *ad libitum* access to feed (dry pellets) and water (one drinking bowl per pen; DRIK-O-MAT, Egebjerg International A/S, Egebjerg Hovedgade 27, Denmark).

Measurement and analysis

Piglets in each pen were weighed individually d 0 (weaning), d 14 (when feeding of experimental diets commenced), d 35, d 77, d 112 and at slaughter (d 147). Feed intake per replicate was recorded weekly. Average daily feed intake (**ADFI**), average daily gain (**ADG**) and feed conversion ratio (**FCR**) were calculated per pen per period. At the end of each period, 8 pigs or 4 pens per treatment with average body weight were sacrificed to evaluate bone mineralization; one femur per pig was removed and frozen at -20°C in order to determine bone ash content. The metacarpals (**MC**) were manually dissected from each foot. A mid-diaphyseal section was cut from the MC using a band saw (Rexon Laser, Rexon Ind. Corp. Ltd.). The Archimedes principle was used to determine the volume of the bone specimen by weighing in air and suspending in distilled water using an OHAUS Scout pro SPU202 balance (OHAUS Corp. NJ, USA). JPEG images of the MC cross section perpendicular to the long axis were taken using a Nikon D70 single lens reflex digital camera (Nikon Corp., Tokyo, Japan). Images were analyzed for cross section area (**CSA**; cm^2) and moment of inertia (**MOI**) calculated for the bone section rotated about the x-axis through the section centroid in cm^2 , using Image J 1.38u (Rasband, W.S., Image J, U. S. National Institutes of Health, Maryland, USA). Metacarpal ash content was determined by drying at 105°C for 12 hours and ashing in a muffle furnace at 650°C for 6 hours. Bone ash samples were dissolved in 10 ml concentrated hydrochloric acid and diluted to 500 ml with deionised water. Samples were analyzed for Ca and P using MPX inductive coupled plasma (**ICP**) techniques.

Area bone mineral density (**aBMD**; g/cm^2) was measured using dual energy X-ray absorption (**DXA**) with a Hologic QDR 4500 (Hologic, Bedford MA 01730 USA). The feet and MC were scanned in a dorso-palmar position and analyzed using the spinal application.

Pigs were slaughtered at 147 days of age. They were transported 107 km to the abattoir and killed by bleeding after CO_2 stunning. Carcass weight was estimated by multiplying by 0.98

the weight of the hot eviscerated carcass, (minus tongue, bristles, genital organs, kidneys, flare fat and diaphragm) 45 minutes after slaughter. Dressing percentage was calculated as (carcass weight / live weight at harvest) x 100.

Calculation and statistics

Data were analyzed using the GLM procedures of SAS (SAS Inst. Inc., Cary, North Carolina) for a randomized complete block design. The pen pair (n=192) was the experimental unit for analysis of animal performance where the model included the effects of block (n=14), treatment (n=7), sex (n=2) and the interaction effects of treatment and sex. Pig weight at day 14 post weaning was used as a covariate for the analysis of ADFI, ADG and FCR. Carcass weight was used as a covariate for the analysis of carcass quality parameters. The pig (n=56) was the experimental unit for the analysis of bone mineralization, DXA scan data, MC cross sectional area, MC cross sectional MOI and apparent MC density. Pig weight at the corresponding time point was used as covariate in this analysis. No interaction effects were observed and only the effects of treatment and sex are presented here. The results are presented as least squares means \pm SEM. Duncan's Multiple Range procedure was used for means separation. Significant differences were accepted if $P < 0.05$. Furthermore, the effect of reformulation (PC vs. NC1; NC2; NC3), mec addition (NC1+; NC2+; NC3+ vs. NC1; NC2; NC3) and mec reformulation (PC vs. NC1+; NC2+; NC3+) were tested using orthogonal contrasts. Finally, reformulation and mec interaction was evaluated.

Linear regression equations predicting animal performance were established from the chemical composition and nutrient content of diets. Feeding stage was taken into account according to a covariance procedure with physiological stage as a fixed effect. Statistical analysis was performed using PROC GLM (SAS Inst. Inc., Cary, North Carolina). The equations with the lowest residual standard deviations (RSD) are presented.

RESULTS

Diets

The ingredient composition and chemical analysis of the experimental diets is presented in Table 1 and was in agreement with tabulated values for crude protein, crude fat and phosphorus content. Xylanase recovery averaged 133% (ranging from 113 to 158). Phytase recovery averaged 95% (ranging from 70 to 113). These recoveries were within acceptable limits, taking into account the analytical standard deviation and the errors inherent with mec application and feed sampling. Results were analyzed according to theoretical values of recovery expressed in percentage of expected value.

Animal performance

The effect of treatment on pig performance is shown in Table 2. All animals remained healthy throughout the experiment. Animal performance was significantly affected by energy and mineral depletion. The most important effect was observed for intake with a reduction of 38% for NC3 compared with PC over the complete experimental period ($P < 0.001$). Feed intakes for NC1 and NC2 were intermediate and similar ($P = 0.98$). In addition, the overall effect was not linear over the entire feeding period. Feed intake was not affected by diet reformulation from 14 to 35 days ($P = 0.34$) but between d35 and slaughter it was effected by reformulation ($P < 0.001$). The mec restored feed intake ($P < 0.001$) of all reformulated diets up to that found for the PC for the overall feeding period. Last statement was demonstrated by the P value for restoration ($P = 0.18$). Similarly, with digestible phosphorus depletion, the most pronounced effect was observed for later feeding stages (mec; $P < 0.01$). Overall feed intake was predicted using covariance analysis involving digestible phosphorus content, mec addition and interaction ($R^2 = 0.50$, $RSD = 253\text{g}$; $P < 0.001$)

Reducing the dietary content of NE, CP, Ca and dig P was associated with reduced feed intake which consequentially resulted in a reduction in live and carcass weights. Animal

body weight at slaughter was related with NE intake for control diets (i.e. PC, NC1, NC2 and NC3; $R = 0.91$, $RSD = 10\text{kg}$). Thus, pigs fed the NC3 diet had the lowest ($P < 0.001$) live weights and ADG throughout the the experiment and the lowest ($P < 0.001$) carcass weight compared with other groups (Table 2). The most pronounced differences were observed for the last feeding program period (Day 112 to slaughter). The final body weight of pigs fed NC1 and NC2 were intermediate between PC and NC3 ($P < 0.001$). Despite NC2 having lower NE levels compared to NC1, no significant differences was observed for animal BW or ADG. The mec significantly increased animal BW and ADG ($P < 0.001$). In common with the reformulation effect, most important effects were observed during the final feeding period (day 112 to slaughter). The final LW of pigs fed mec-supplemented diets averaged 113 kg and ranged from 108 – 116kg. Interactions between mec and diet were not significant. The final body weight of mec supplemented pigs remained significantly below corresponding values obtained for PC diet.

Feed efficiency was significantly affected by both reformulation and MEC addition for all feeding program phases. Pigs on NC3 had a ~8.5% poorer FCR than those on the PC averaged from day 14 to slaughter ($P < 0.001$). Significant improvements in FCR were achieved using mec ($P < 0.001$) with average (min-max) 2.6 (1.7 – 3.8) %. Minimum improvement were achieved for NC3 whereas maximum improvement were achieved for NC2. Reformulation and mec interaction was not significant and remained lower than impairment. The FCR of mec-supplemented diets for the overall experiment was higher by 2.2%, 2.6% and 6.7% for NC1, NC2 and NC3 supplemented diets, respectively compared with PC.

Bone mineralization

Bone parameters are presented in Table 3. In connection with growth and deficiency establishment, no significant effects of either reformulation or mec were observable up to day

77. The first parameter effected in the present study was the apparent bone mineral density for days 77, 112 and at slaughter. Apparent bone mineral density decreased in association with mineral reformulation, with highest values observed for PC fed pigs at all growth stages with a linear decrease found for NC diets. Parameter was linearly related (i.e. PC, NC1, NC2 and NC3) with P or Ca intake ($R^2 = 0.76$, RSD 0.04, $P < 0.001$). The mec addition fully restored parameters to PC control level ($P > 0.05$). The effect of Ca and P restriction was observed at day 77 for foot aBMD while the effect of restriction on aBMD in the Metacarpel III was not noticed until day 112, thereby indicating a greater sensibility for foot aBMD to mineral restriction. No effect of reformulation or mec was observed at the moment of inertia except at day 77 whereas change in bone density might suggest change in bone breakdown susceptibility for each measurement.

Bone mineral content is presented in Table 4. The principle effects observed were for metacarpal DM content at day 77 and at slaughter ($P < 0.001$). Dietary P and Ca restriction reduced metacarpal DM content in a linear manner. The mec successfully restored metacarpal DM content at slaughter to the level of PC when added either to NC1, NC2 and NC3 diets.

DISCUSSION

Evidence of P deficiency in this experiment was obvious. Voluntary feed intake reduced as the P content in the diet decreased which is in agreement with previous data for growing finishing pigs (Harper *et al.*, 1997; Matsui *et al.*, 2000; Braña *et al.*, 2006). The ADFI was not influenced by treatment up to day 35 of age and it is likely that there is a lag phase before a deficiency induces changes in appetite Kim *et al.* (2005). Kim *et al.* did not find any effect of mineral depletion on FCR. The fact that we did in the current study is most likely due to the concomitant energy and mineral restriction, whereas Kim *et al.* (2005) only looked at the effect of different phosphorus levels. Coupled with the P effect on ADFI,

restricting NE and CP in the NC1, NC2 and NC3 diets resulted in a reduction in BW and ADG of pigs relative to the PC diet. This reduction was seen 3 weeks after feeding the experimental diets commenced, demonstrating that even minor reductions in NE, CP, Ca and P can rapidly reduce weight gain in pigs and reduce weight gain to slaughter. In addition, reduction of the ratio P:Ca might also exacerbate the effects of the available P deficiency (Vipperman *et al.*, 1974; Liu *et al.*, 1998; Brady *et al.*, 2002, Wu *et al.*, 2018) and support the reduction of animal performance among NC diets. In the present study, FCR was highly correlated with digP and Ca content of the diet for unsupplemented PC or NC diets ($R^2 = 0.80$; $P < 0.001$). These results contrast with those of Woyengo *et al.* (2008) using a higher total P constraint (0.48% vs 0.53 % for PC diet) and smaller reformulation specification (-0.04 vs -0.10 percentage unit). Thus, it should be noted that a reduction in the dietary concentration of available P will limit pig growth (i.e. feed intake and body weight gain) only when the reduction is sufficient to do so (Braña *et al.*, 2006; Létourneau-Montminy *et al.*, 2012). In these last study (Braña *et al.*, 2006) where a reduction in dietary available P resulted in reduced pig growth (i.e. feed intake and body weight gain), the dietary available P in the basal diet was lower than that recommended by NRC (1998) by at least 0.1 percentage unit. Such reduction was possible because the diets were based on corn, which is low in available P (0.08%; NRC, 1998).

Hinson *et al.* (2009) found similar results for bone ash to those of the present study. Phosphorus depletion (-11 g/kg) without Ca reduction resulted in the lowest detrimental effect on bone ash content (-3.1%) and highlights the relationship between Ca and P for bone metabolism (Létourneau-Montminy *et al.*, 2012). The demineralization of bone observed in the present study as a result of an insufficient supply of P in pigs is in agreement with the results of other studies (Pointillart 1991; Carter *et al.*, 1996; Harper *et al.*, 1997; Mc Cormick *et al.*, 2017).

Numerous parameters were measured for either bone mineralization or bone quality in the present study. Phosphorus and Ca contents were positively related in bone, suggesting important effects of ratio in the mineralization step. Bone ash content and metacarpal DM were negatively related with Ca bone content, which would suggest an organic matrix development concomitant with bone mineralization and subsequently more water attraction. All bone quality parameters except cross section area and moment of inertia were positively related with Ca bone content. These results were in agreement with results extracted from human food research (Fehily et al., 1992; Metz et al., 1993). Therefore, some parameters remained unchanged despite the dietary mineral restriction such as Cross section area, Bone ash content or Ca content in ash. No significant difference was observed and stressed low sensibility of such measurement.

Supplementation of NC diets with a carbohydrase/phytase combination improved feed efficiency feed intake and body weight gain. Despite the presence of numerous reports describing the effects of phytase and carbohydrases, alone or in combination, in various cereal based poultry diets (Rosen, 2004), little data is available concerning the use of carbohydrase and phytase combinations in pig diets. A meta-analysis using published data (Nortey et al., 2007a; Lindberg et al., 2007; Moehn et al., 2007; Kim et al., 2008; Woyengo et al., 2008; Atakora et al., 2011; Yanez et al., 2011) found an additive effect for both enzymes for nutrient digestibility and pig growth (unpublished data). As a result, the current discussion will focus first on phytase and then on carbohydrase. Increasing dietary available P, by increasing total P inclusion in the diet (Veum *et al.*, 2007) and by increasing P availability by adding phytase to a reduced P diet (Harper *et al.*, 1997) has been shown to stimulate appetite in pigs.

Improved FCR was associated with greater nitrogen retention because of the associated water deposition (Just, 1984). A previous meta-analysis based on 12 diets

(unpublished data) suggested an increase of 11.9%, 8.1% and 1.5% units for P, Ca and N digestibility, respectively in response to phytase. Part of this improvement might be related to phytase's direct effect on P digestibility (Simons *et al.*, 1990; Jongbloed *et al.*, 1991; Lei *et al.*, 1991) but also due to its effect on the digestibility of other nutrients such as protein as well as reduction in endogenous losses (Kornegay and Quian, 1996; Yi *et al.*, 1996) as phytic acid acts as a Ca- and protein-binding agent. Phytase digestibility effect was completed by carbohydrase, as it is reasonable to state that both xylans and β -glucans participate to non-starch polysaccharide networks and subsequent unstirred layers at the mucosal surface breakdown (Carpita and Gibeaut, 1993). Cell wall disruption by carbohydrase results in a more rapid access by endogenous digestive enzymes to the endosperm cell content (Nortey *et al.*, 2007 b; 2008). Furthermore, xylanase and the range of carbohydrases contained in the multi enzyme complex, also increases the nutritional value of feed (Emiola *et al.*, 2009) due to the breakdown of non-starch polysaccharides (NSP) in the cell wall of wheat (Nortey *et al.*, 2008) thereby making more energy, amino acids and P available to the pig (Barrera *et al.*, 2004; Nortey *et al.*, 2008). As a result, improved growth (Cromwell *et al.*, 1991; Lei *et al.*, 1991) and improved feed efficiency (Cromwell *et al.*, 1991, Yang *et al.*, 2017) have been reported due to microbial phytase and/or carbohydrase addition to low-P diets. However as the improvements in growth in the current trial did not result in live weights similar to those fed PC diets which were formulated to meet NRC minimum requirements (NRC 1998). The latter suggests that reductions in dietary nutrient and mineral density equal or greater than those in this current trial would reduce pig growth.

The effect of phytase supplementation to low P pig diets on metacarpal, metatarsal and femur quality has previously been widely studied in the past (Cromwell *et al.*, 1995; Veum, 1996; O'Quinn *et al.*, 1997; Hinson *et al.*, 2009; Kühn *et al.*, 2016) and rib bone (Harper *et al.*, 1997). Metacarpal and foot aBMD quickly responded to available nutrients and minerals (i.e.

Ca and P). The current study found no effect of dietary constraint on metacarpal DM content and bone quality parameters in pigs up to 77 days of age. This suggests that the pigs had built up a resilience capacity to face P and Ca deficiency. In addition, the fact that that metacarpal aBMD was maintained for up to 112 days while feeding low nutrient and mineral density diets emphasizes the importance of this reserve. This pattern was seen in pigs fed all diets and specifically in NC3, where available *P* was reduced by 50% in addition to reductions in energy and protein, which represents a very severe reduction. Where the MEC was supplemented it prevented a reduction in metacarpal aBMD (e.g. NC3+mec had a similar metacarpal aBMD value to PC pigs). Numerous studies have found that most bone development occurs in early life (Greene *et al.* 2005; Rizzoli *et al.* 2009). This suggests that the reserve capacity had the potential to withstand a large nutritional insult, but that eventually the reserve is exhausted.

CONCLUSION

Feeding a sequence of reduced digestible energy, crude protein, calcium and digestible phosphorus diets decreased body weight, feed intake and daily gain compared to pigs fed a sequence of diets formulated according to the recommendations of NRC (1998). The primary effect was a feed intake reduction. Supplementing mineral and nutrient deficient diets with a combination of phytase and carbohydrases increased feed intake, daily gain and pig weight above that found with un-supplemented diets. Growth performance did not reach levels observed with the NRC (1998) adequate diet. Results from dual energy X-ray absorptiometry showed that multi enzyme complex supplementation to nutrient and mineral deficient diets increased foot and metacarpal bone mineral densities up to levels found for pigs fed NRC (1998) adequate diets.

It is concluded that the addition of the multi enzyme complex to pig diets can allow diets to be formulated with lower digestible energy, amino acids, calcium and digestible phosphorus than currently recommended. The benefits for the producer include reduced feed costs and reduced nutrient excretion.

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ACCEPTED MANUSCRIPT

Table 1: Ingredient Composition and Chemical Analysis of Experimental Diets

	Post weaning 2				Growing 1				Growing 2				Finishing			
	PC	NC1	NC2	NC3	PC	NC1	NC2	NC3	PC	NC1	NC2	NC3	PC	NC1	NC2	NC3
Composition, g/kg																
Wheat	0.0	0.0	0.0	0.0	150.0	154.4	149.0	103.0	150.0	100.0	100.0	80.0	180.6	150.6	115.0	144.9
Corn	553.2	583.8	603.1	603.6	477.5	450.0	450.0	500.0	456.6	514.6	508.4	540.1	501.3	528.2	550.0	550.0
Soybean meal hipro	268.0	256.1	282.2	283.0	234.6	221.5	216.9	224.7	108.9	138.8	133.7	144.0	77.7	85.4	81.6	101.1
Wheat bran	0.0	0.0	0.0	0.0	65.4	100.0	100.0	79.8	150.0	129.1	130.9	96.4	164.7	134.0	144.5	58.3
Molasses	0.0	0.0	0.0	0.0	40.0	40.0	40.0	40.1	40.0	40.1	40.1	60.0	50.1	50.0	50.1	60.0
Soy full fat	100.0	100.0	51.2	50.0	0.0	0.0	0.0	0.0	66.8	30.1	30.3	30.0	0.0	0.0	0.0	0.0
Oil	14.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lys	3.7	3.8	3.9	3.9	5.5	5.5	5.4	5.3	5.3	5.1	5.1	5.0	5.3	5.2	5.2	4.9
Met	1.6	1.6	1.6	1.6	1.7	1.7	1.7	1.7	1.4	1.4	1.4	1.4	1.0	1.1	1.1	1.1
Thr	1.5	1.4	1.5	1.5	2.2	2.2	2.2	2.1	2.0	1.9	1.9	1.9	1.9	1.8	1.8	1.7
Trp	0.5	0.5	0.5	0.5	0.7	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6

Dicalcium phosphate	18.0	10.7	11.0	8.0	9.5	1.3	1.4	0.0	4.9	0.0	0.0	0.0	2.4	0.0	0.0	0.0
Calcium carbonate	12.9	15.0	15.0	15.6	6.9	9.4	9.4	9.1	7.5	8.1	8.1	6.9	8.4	7.4	7.4	6.2
Sepiolite	20.0	21.1	24.0	26.3	0.0	7.4	17.4	27.6	0.0	24.2	33.5	47.7	0.0	29.7	36.7	65.2
Salt	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Premix ¹	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0

Nutrient content, %

Dry matter ²	89.5	89.05	89.4	89.5	88.5	88.8	88.75	88.4	86.8	87.15	87.35	87.5	86.1	86.55	87.15	89.4
Crude Protein ²	21.3	21	20.35	19.8	18.5	17	17.2	17.45	15.4	15.65	15.3	14.6	13.1	12.4	13.2	13.15
Crude fat ²	6.2	5	4.2	4.3	3.2	3.3	3.25	3.25	4.5	3.8	3.65	3.65	3.4	3.15	3.25	2.7
Crude fiber ²	2.6	2.65	2.55	2.45	2.5	2.85	2.8	2.55	3.3	3.1	3.0	2.55	3.4	3.1	3.0	2.2
Ash ²	7.5	7.2	7.15	7.5	4.8	5.0	6.0	6.6	4.6	6.2	6.9	7.85	3.9	6.3	7.0	9.25
Ca ²	0.96	0.92	0.90	0.91	0.60	0.50	0.55	0.53	0.59	0.49	0.51	0.49	0.59	0.49	0.48	0.44
P ²	0.75	0.58	0.55	0.50	0.48	0.37	0.37	0.34	0.43	0.33	0.34	0.31	0.38	0.33	0.33	0.30
NE ³ , MJ/kg	9.72	9.50	9.36	9.36	9.32	9.18	9.09	9.09	9.31	9.14	9.04	9.04	9.28	9.13	9.04	9.02
Dig Lys ³	1.26	1.24	1.23	1.23	1.18	1.15	1.13	1.13	1.00	0.98	0.98	0.97	0.82	0.82	0.81	0.81
Dig P ³	0.38	0.26	0.26	0.21	0.28	0.16	0.16	0.12	0.24	0.14	0.14	0.12	0.20	0.14	0.14	0.10

¹ Premix composition (starter/growerI / growerII/finisher) 620/60 g/ton Copper sulphate; 450/120 g/ton ferrous sulphate monohydrate; 60/40 g/ton manganese oxide; 150/100 g/ton zinc oxide; 1.0/0.5 potassium iodate; 0.6/0.4 g/ton sodium selenite; 6/2 iu /ton Vitamin A; 1.0/0.5 miu/ton Vitamin D3; $100 \times 10^3 / 40 \times 10^3$ iu/ton Vitamin E; 4/4 g/ton Vitamin K; 15/15 mg/ton Vitamin B12; 2.0/2.0 g/ton Riboflavin; 12/12 g/ton Nicotinic acid; 10/10 g/ton pantothenic acid; 250/0 g/ton choline chloride; 2/2 g/ton VitaminB1; 3/3 g/ton Vitamin B6/60 g/ton endox; 155/15 g/ton Copper; 90/24g/ton Iron; 47/31 g/ton Manganese; 120/80 g/ton Zinc; 0.6/0.3 g/ton Iodine; 3/0.2 g/ton Selenium.

² Analysed values

³ Calculated from tabulated values (Sauvant et al., 2004)

ADFI (g)	1839ab	1647bc	1826ab	1684c	1885a	1624c	1786abc	0.007	<0.001	0.892	259	<0.001
ADG (g)	847a	678dc	780ab	661d	828a	523e	743bc	<0.001	<0.001	0.025	110	<0.001
FCR ² (g/g)	2.21c	2.51cb	2.38cb	2.62b	2.27cb	3.26a	2.43cb	<0.001	<0.001	0.221	0.48	<0.001
Day 77 - 112												
ADFI (g)	2486a	1854bc	2199ab	1878bc	2213ab	1698c	2472a	<0.001	<0.001	0.174	478	<0.001
ADG (g)	975a	587d	818b	516d	842b	438e	718c	<0.001	<0.001	<0.001	138	<0.001
FCR ² (g/g)	2.57c	3.44ab	2.75bc	3.91a	2.65bc	4.10a	3.61a	<0.001	<0.001	0.112	0.99	<0.001
Day 112 - slaughter												
ADFI (g)	3065ab	2018c	2641b	1802dc	3060ab	1612d	3184a	<0.001	<0.001	0.497	398	<0.001
ADG (g)	866a	514b	776a	465cb	799a	369c	897a	<0.001	<0.001	0.474	137	<0.001
FCR ² (g/g)	3.57	3.95	3.57	4.06	3.88	4.79	3.56	0.036	0.013	0.886	0.93	0.160
Day 14 -slaughter												
ADFI (g)	1868a	1721bc	1771abc	1679c	1842ab	1640c	1894a	<0.001	<0.001	0.180	220	<0.001
ADG (g)	705a	622bc	654ab	595c	676ab	578c	667ab	<0.001	<0.001	<0.001	68	<0.001
FCR ² (g/g)	2.69b	2.81ab	2.75ab	2.87ab	2.76ab	2.92ab	2.87a	0.001	0.026	0.082	0.32	0.003

¹ Data set (n = 192) was analyzed by variance analysis using treatment as fixed effect (n = 7). Orthogonal contrast reformulation (PC vs. NC diets), mec (NC vs NC + mec) and restoration (PC vs NC + mec diets) were tested. Means with the same letters within the row do not differ significantly (P>0.05)

² FCR – Feed conversion efficiency

Table 4 : Effect of Dietary treatment on Bone Mineralization in the pig at Different Growth Stages up to Slaughter.

Constraint	PC		NC1		NC2		NC3		Statistic ¹			se	P-value
	Contrast effect												
	Mec	-	-	+	-	+	-	+	Reform	Mec	Restoration		
Bone Ash (g/kg metacarpal DM)													
Day 35	451	411	441	438	446	409	415	0.265	0.395	0.389	14	0.76	
Day 77	465	427	454	427	472	413	447	0.165	0.104	0.787	18	0.68	
Day 112	498ab	411dc	470ab	390cd	519a	372d	443bc	0.089	0.013	0.706	18	<0.01	
Day of slaughter	596	471	545	464	554	453	614				22	0.11	
Ca content in ash (g/kg)													
Day 35	469	495	482	460	478	521	476	0.343	0.368	0.567	12	0.09	
Day 77	405	438	412	440	424	478	411	0.010	0.005	0.493	10	0.07	
Day 112	401	401	385	405	384	436	415	0.76	0.527	0.819	23	0.96	
Day of slaughter	322	393	397	371	367	427	371	0.913	0.210	0.266	17	0.42	
P content in ash (g/kg)													
Day 35	204ab	199ab	215a	190b	204ab	204ab	190b	0.065	0.370	0.060	7	<0.05	

Day 77	202	205	191	202	213	234	213	0.604	0.622	0.847	14	0.82
Day 112	204	178	175	186	194	192	205	0.446	0.732	0.477	13	0.78
Day of slaughter	178	181	196	179	175	187	183	0.265	0.074	0.898	10	0.81
Metacarpal DM (g/kg)												
Day 35	717	665	729	740	709	704	710	0.657	0.517	0.954	17	0.39
Day 77	706a	658b	700a	679ba	702a	614c	679ba	<0.001	<0.001	0.466	11	<0.001
Day 112	708	688	703	675	707	662	687	0.107	0.128	0.521	11	0.66
Day of slaughter	758a	676b	758a	668b	770a	656b	770a	0.002	<0.001	0.753	10	<0.001

¹Data set (n = 192) was examined through variance analysis using treatment as fixed effect (n = 7). Orthogonal contrast reformulation (PC vs. NC diets), mec (NC vs NC + mec) and restoration (PC vs NC + mec diets) were tested. Means with the same letters within the row do not differ significantly (P>0.05)

Table 3 : Effect of Treatment on Bone Quality at Different Growth Stages of Pig growth up to Slaughter

Constraint	Statistic ¹											
	PC	NC1		NC2		NC3		Contrast effect				
		-	-	+	-	+	-	+	Reform	Mec	Restoration	Se
Metacarpal III aBMD² (g/cm²)												
Day 35	0.255	0.24	0.259	0.249	0.244	0.227	0.235	0.174	0.293	0.300	0.006	0.06
Day 77	0.303	0.231	0.287	0.292	0.259	0.302	0.284	0.420	0.950	0.385	0.023	0.38
Day 112	0.422a	0.280c	0.391ab	0.309c	0.413a	0.268c	0.357b	<0.001	<0.001	0.052	0.014	<0.01
Day of slaughter	0.471a	0.345bc	0.455a	0.375b	0.466a	0.304c	0.436a	<0.001	<0.001	0.263	0.018	<0.01
Foot aBMD² (g/cm²)												
Day 77	0.401a	0.301d	0.396b	0.320cd	0.360b	0.262e	0.325c	<0.001	<0.001	<0.001	0.02	<0.001
Day 112	0.458a	0.349bc	0.425a	0.326bc	0.424a	0.312c	0.368b	<0.001	0.008	0.018	0.016	<0.05
Day slaughter	0.491a	0.380c	0.474a	0.424b	0.488a	0.348c	0.464a	<0.001	<0.001	0.375	0.014	<0.01
Cross section area (cm²)												
Day 35	0.599	0.801	0.617	0.661	0.65	0.693	0.708	0.140	0.222	0.291	0.039	0.37
Day 77	1.01	0.81	0.92	0.86	1.07	0.61	0.85	0.019	0.025	0.480	0.068	0.14

Day 112	0.916	0.751	0.812	0.98	0.829	0.92	0.84	0.727	0.427	0.178	0.049	0.38
Day slaughter	1.429	1.371	1.281	1.493	1.33	1.18	1.229	0.674	0.594	0.242	0.049	0.43
Moment of Inertia (cm⁴)												
Day 35	0.073	0.114	0.079	0.08	0.081	0.089	0.097	0.284	0.469	0.365	0.010	0.57
Day 77	0.214	0.151	0.173	0.171	0.234	0.082	0.148	0.020	0.056	0.320	0.022	0.10
Day 112	0.145	0.117	0.124	0.167	0.111	0.165	0.116	0.882	0.204	0.238	0.017	0.59
Day slaughter	0.418	0.574	0.359	0.645	0.438	0.392	0.306	0.406	0.077	0.587	0.057	0.06
Apparent density (g/cm³)												
Day 35	1.35	1.28	1.33	1.3	1.34	1.32	1.32	0.127	0.148	0.350	0.02	0.63
Day 77	1.40a	1.25d	1.37ab	1.31c	1.39a	1.22d	1.32bc	0.002	0.006	0.230	0.02	<0.01
Day 112	1.41ab	1.30c	1.40ab	1.29c	1.48a	1.24c	1.38b	0.018	<0.001	0.452	0.03	<0.01
Day slaughter	1.56ab	1.42cd	1.49bc	1.39d	1.56ab	1.37d	1.63a	<0.001	<0.001	0.730	0.03	<0.001

¹Data set (n = 192) was examined through variance analysis using treatment as fixed effect (n = 7). Orthogonal contrast reformulation (PC vs.

NC diets), mec (NC vs NC + mec) and restoration (PC vs NC + mec diets) were tested. Means with the same letters within the row do not differ significantly (P>0.05)

²Metacarpal III aBMD: Metacarpal III apparent Bone Mineral Density; Foot aBMD: Foot apparent Bone Mineral Density