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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 carbohydrolase (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 carbohydrolase 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, carbohydrolase
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 carbohydrolase 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 carbohydrolase (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 carbohydrolases 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 ± 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 (1.81 m x 1.18 m) 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; cm²) and moment of inertia (MOI) calculated for the bone section rotated about the x-axis through the section centroid in cm², 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/cm²) 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 CO₂ 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 ± 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 ($R^2 = 0.50$, $RSD = 253g; P < 0.001$)

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; \( R = 0.91, \) \( \text{RSD} = 10\text{kg} \)). Thus, pigs fed the NC3 diet had the lowest \( (P < 0.001) \) live weights and ADG throughout 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 – 116 kg. 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 (R² = 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 carbohydrolase/phytase combination improved feed efficiency feed intake and body weight gain. Despite the presence of numerous reports describing the effects of phytase and carbohydrolases, alone or in combination, in various cereal based poultry diets (Rosen, 2004), little data is available concerning the use of carbohydrolase 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 carbohydrolase. 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 carbohydrolase, 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 carbohydrolase results in a more rapid access by endogenous digestive enzymes to the endosperm cell content (Nortey et al., 2007b; 2008). Furthermore, xylanase and the range of carbohydrolases 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 carbohydrolase 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 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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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 | 477.5 450.0 450.0 450.0 | 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 |
| Ingredient              | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  |
|-------------------------|------|------|------|------|------|------|------|------|------|
| Dicalcium phosphate     | 18.0 | 10.7 | 11.0 | 8.0  | 9.5  | 1.3  | 1.4  | 0.0  | 4.9  |
| Calcium carbonate       | 12.9 | 15.0 | 15.0 | 15.6 | 6.9  | 9.4  | 9.4  | 9.1  | 7.5  |
| Sepiolite               | 20.0 | 21.1 | 24.0 | 26.3 | 0.0  | 7.4  | 17.4 | 27.6 | 0.0  |
| Salt                    | 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  |

| Nutrient content, %     |      |      |      |      |      |      |      |      |      |
|-------------------------|------|------|------|------|------|------|------|------|------|
| Dry matter              | 89.5 | 89.05| 89.5 | 88.5 | 88.8 | 88.75| 88.4 | 86.8 | 87.15|
| Crude Protein           | 21.3 | 21.0 | 20.35| 19.8 | 18.5 | 17.2 | 17.45| 15.4 | 15.65|
| Crude fat               | 6.2  | 5.0  | 4.2  | 4.3  | 3.2  | 3.3  | 3.25 | 3.25 | 4.5  |
| Crude fiber             | 2.6  | 2.65 | 2.55 | 2.45 | 2.5  | 2.85 | 2.8  | 2.55 | 3.3  |
| Ash                     | 7.5  | 7.2  | 7.15 | 7.5  | 4.8  | 5.0  | 6.0  | 6.6  | 4.6  |
| Ca                      | 0.96 | 0.92 | 0.90 | 0.91 | 0.60 | 0.50 | 0.55 | 0.53 | 0.59 |
| P                       | 0.75 | 0.58 | 0.55 | 0.50 | 0.48 | 0.37 | 0.37 | 0.34 | 0.43 |
| NE, MJ/kg               | 9.72 | 9.50 | 9.36 | 9.36 | 9.32 | 9.18 | 9.09 | 9.09 | 9.31 |
| Dig Lys                 | 1.26 | 1.24 | 1.23 | 1.23 | 1.18 | 1.15 | 1.13 | 1.13 | 1.00 |
| Dig P                   | 0.38 | 0.26 | 0.26 | 0.21 | 0.28 | 0.16 | 0.16 | 0.12 | 0.24 |
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 mm/ton Vitamin D3; 100*10^7/40*10^7 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)
Table 2: Effect of dietary treatment on pig growth and feed utilization to slaughter

| Constraint         | PC       | NC1      | NC2      | NC3      | Statistics1 |
|--------------------|----------|----------|----------|----------|-------------|
|                    | Reformulation | Mec     | Restoration | se | P-value |
| Pig Weight (kg)    |          |          |          |          |             |
| Day 14             | 12.5     | 12.5     | 12.3     | 12.4     | 12.6        | 12.3       | 12.3       | 0.808     | 0.850     | 0.911     | 1     | 0.959    |
| Day 35             | 24.8ab   | 23.1c    | 24.8ab   | 23.6bc   | 25.2a       | 22.0d      | 24.1abc    | 0.642     | 0.970     | 0.658     | 1     | <0.001   |
| Day 77             | 60.0a    | 52.4c    | 57.4ab   | 51.6c    | 59.4a       | 44.1d      | 55.1bc     | <0.001    | <0.001    | 0.721     | 2     | <0.001   |
| Day 112            | 95.3a    | 73.3d    | 84.9bc   | 70.0d    | 89.8ab      | 62.5c      | 81.7c      | <0.001    | <0.001    | 0.040     | 6     | <0.001   |
| Day of slaughter   | 124.5a   | 91.8c    | 108.4b   | 83.7c    | 116.1b      | 74.6d      | 114.7b     | <0.001    | <0.001    | <0.001    | 8     | <0.001   |
| Cold weight        | 98.9a    | 65.9c    | 83.0b    | 63.3c    | 91.5ab      | 57.6d      | 85.1b      | <0.001    | <0.001    | 0.002     | 12    | <0.001   |
| Day 14 - 35        |          |          |          |          |             |
| ADFI (g)           | 934      | 950      | 1038     | 948      | 991         | 925        | 993        | 0.347     | 0.934     | 0.375     | 53    | <0.001   |
| ADG (g)            | 574ab    | 494cd    | 572ab    | 516bc    | 587a        | 445d       | 539abc     | 0.611     | 0.966     | 0.630     | 108   | <0.001   |
| FCR2 (g/g)         | 1.72b    | 2.07ab   | 1.90ab   | 1.85ab   | 1.71b       | 2.21a      | 1.88ab     | 0.758     | 0.847     | 0.654     | 0.66  | 0.070    |
| Day 35 - 77        |          |          |          |          |             |
|    | ADFI (g) | ADG (g) | FCR² (g/g) |
|----|---------|---------|------------|
| Day 77 - 112 | 1839ab | 1647bc | 1826ab | 1684c | 1885a | 1624c | 1786abc | 0.007 | <0.001 | 0.892 | 259 | <0.001 |
|  | 847a | 678dc | 780ab | 661d | 828a | 523e | 743bc | <0.001 | <0.001 | 0.025 | 110 | <0.001 |
|  | 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 112 - slaughter | 2486a | 1854bc | 2199ab | 1878bc | 2213ab | 1698c | 2472a | <0.001 | <0.001 | 0.174 | 478 | <0.001 |
|  | 975a | 587d | 818b | 516d | 842b | 438e | 718c | <0.001 | <0.001 | <0.001 | 138 | <0.001 |
|  | 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 14 - slaughter | 3065ab | 2018c | 2641b | 1802dc | 3060ab | 1612d | 3184a | <0.001 | <0.001 | 0.497 | 398 | <0.001 |
|  | 866a | 514b | 776a | 465cb | 799a | 369c | 897a | <0.001 | <0.001 | 0.474 | 137 | <0.001 |
|  | 3.57 | 3.95 | 3.57 | 4.06 | 3.88 | 4.79 | 3.56 | 0.036 | 0.013 | 0.886 | 0.93 | 0.160 |

ADF (g) 2 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 
FCR² (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 
ADF (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 
FCR² (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 
ADF (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 
FCR² (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 
ADF (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 
FCR² (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
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)

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 | Statistic1 |
|------------|-----|-----|-----|-----|------------|
| Mec        | -   | -   | +   | -   | +          |
|            | Reform | Mec | Restoration | se | P-value |
| 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 | 411bc | 470ab | 390d | 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 |

1: Statistic values are calculated using ANOVA.
|               | Day 77 | Day 112 | Day of slaughter |
|---------------|--------|---------|-----------------|
| Metacarpal DM (g/kg) |        |         |                 |
| Day 35        | 717    | 665     | 729             |
| Day 77        | 706a   | 658b    | 700a            |
| Day 112       | 708    | 688     | 703             |
| Day of slaughter | 758a  | 676b    | 758a            |

*Data set (n = 192) was examined through variance analysis using treatment as fixed effect (n = 7). Orthogonal contrast reformulation (PC vs. NC diets), mc (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                | Metacarpal III aBMD² (g/cm²)              | Foot aBMD² (g/cm²)              | Cross section area (cm²) |
|---------------------------|------------------------------------------|--------------------------------|--------------------------|
|                           | Statistic†                              | Statistic†                    |                          |
|                           | Contrast effect                          | Reform Restor. Se P-value      |                          |
|                           |                                          |                              |                          |
| Mec                       |                                          |                              |                          |
|                           | PC NC1 NC2 NC3                           | PC NC1 NC2 NC3                | PC NC1 NC2 NC3           |
|                           |                                          |                              |                          |
|                             | Day 35                                  | 0.255                        | 0.24 ± 0.259             | 0.249 ± 0.244            | 0.227 ± 0.235            | 0.174 ± 0.293 | 0.300 ± 0.006 | 0.006 ± 0.06 |
|                             | Day 77                                  | 0.303                        | 0.23 ± 0.287             | 0.292 ± 0.259            | 0.302 ± 0.284            | 0.420 ± 0.950 | 0.385 ± 0.023 | 0.038 ± 0.38 |
|                             | Day 112                                 | 0.422a ± 0.391abc            | 0.309c ± 0.413a           | 0.268c ± 0.357b          | <0.001 ±<0.001           | 0.052 ± 0.014 | 0.014 ± <0.01 |
|                             | Day of slaughter                        | 0.471a ± 0.455b              | 0.375b ± 0.466a           | 0.304c ± 0.436a          | <0.001 ±<0.001           | 0.263 ± 0.018 | 0.018 ± <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 ± 0.37  |
|                             | Day 77                                  | 1.011                        | 0.81 ± 0.92               | 0.86 ± 1.07              | 0.61 ± 0.85              | 0.019 ± 0.025 | 0.480 ± 0.068 | 0.14 ± 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 (cm^4)

| Day   | 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 35 | 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 77 | 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 112 | 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/cm^3)

| Day   | 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 35 | 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 77 | 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 112 | 1.56ab | 1.42cd | 1.49bc | 1.39d | 1.56ab | 1.37d | 1.63a | <0.001 | <0.001 | 0.730 | 0.03 | <0.001 |

1 Data 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)

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