**The effect of 25-hydroxyvitamin D3 and phytase inclusion on pig performance, bone parameters and pork quality in finisher pigs**

**Running Title:** Combination of 25-hydroxyvitamin D3 and phytase in pig diet

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**Summary**

The objective of the present study was to investigate the effects of supplementing both phytase and 25-hydroxyvitamin D3 (25-OH-D₃) on pig performance, nutrient digestibility, carcass characteristics, bone parameters and pork quality in finisher pigs. The experimental design was a 2 × 2 factorial comprising of four dietary treatments. One hundred and twenty pigs (60 male, 60 female) were blocked according to live weight and sex and allocated to the following dietary treatments: low P (4.81 g/kg) diet (basal) (T1); low P diet + phytase (T2); low P diet + 25-OH-D₃ (T3) and low P diet + phytase + 25- OH-D₃ (T4). Pigs supplemented with phytase had a lower average daily feed intake (ADFI) (2.45 kg v. 2.59 kg; *P <* 0.05) and lower feed conversion ratio (FCR) (2.74 kg/kg v. 2.85 kg/kg; *P <* 0.05) compared to pigs offered the non-phytase diets. Pigs offered phytase diets had a higher (*P <* 0.05) coefficient of apparent total tract digestibility (CATTD) of ash, phosphorous (P) and calcium (Ca) compared with pigs offered the non-phytase supplemented diets. Pigs offered the 25-OH-D3 diets had a higher CATTD of N and ash. Pigs offered the phytase diets had increased (*P <* 0.05) bone DM, ash, Ca, P and density compared to the non-phytase diets. There was a significant interaction (*P <* 0.05) between phytase and 25-OH-D3 on cook loss %. Pigs offered 25-OH-D3 had increased cook loss % values over the basal diet, however there was no effect on cook loss % when phytase was added. Pigs offered 25-OH-D3 exhibited higher (*P <* 0.05) WBSF values and lower (*P <* 0.05) pork lightness (L\*) surface colorimeter values. In conclusion, there was no benefit to offering a combination of phytase and 25-OH-D3 on pig performance, bone parameters or pork quality.

**Keywords**

Phytase, 25-hydroxvitamin D₃, bone parameters, pork quality, phosphorus, pigs

**1 Introduction**

Cereal based pig diets require supplementation of inorganic phosphorus (P), as almost two thirds of the total P is present in the form of phytate, which is poorly utilised by monogastric animals due to low phytase activity in the digestive tract (Brady, Callan, Mcgrane & O’Doherty, 2002; Humer, Schwarz & Schedle, 2015). The inclusion of phytase enzymes in low P pig diets can reduce the anti-nutritional effect of phytate and improve the digestibility of minerals and nutrients and reduce the negative impact of inorganic P excretion to the environment (Shim, Chae & Lee, 2004; Singh & Satyanarayana, 2015). Leg weakness is evident in fast-growing swine production systems (Stern, Lundeheim, Johansson & Andersson, 1995) and can affect the thriftiness of fattening pigs. Leg weakness has also been correlated to increased culling percentage in breeding swine (Serenius & Stalder, 2006; Quinn, 2012). The supplementation of phytase in low P diets in grower-finisher pigs has improved skeletal bone mineralisation (O'Doherty, Gahan, O’Shea, Callan & Pierce, 2010). However, it has also been reported that maximum bone mineralisation levels are achieved at higher P levels than are required for optimum gain and feed utilisation (NRC, 2012).

Another major factor affecting efficient Ca and P absorption level for bone mineralisation is adequate vitamin D status (Holick, 2006). The supplementation of pig diets with 25-hydroxyvitamin D₃ (25-OH-D₃) has a major advantage over vitamin D₃ as it provides five times the relative biological value of total vitamin D (Cashman et al., 2012), making the biologically active metabolite 25-OH-D3 more available. There is a paucity of research available investigating the benefits, if any, of combining phytase and 25-OH-D3 on bone mineralisation and performance in finishing pigs.

Previous research in beef cattle has shown that the supplementation of vitamin D₃ has the potential to elevate muscle Ca concentrations, enhance the level of post-mortem proteolysis and improve beef tenderness (Swanek et al., 1999; Montgomery et al., 2000). The supplementation of phytase and 25-OH-D3 separately has been shown to improve Ca and P retention (Qian, Kornegary & Denbow 1997; Dersjant-Li, Awati, Schulze & Partridge 2015), which in turn may increase muscle Ca concentrations and improve post-mortem proteolysis, thereby increasing pork tenderness. However, there is very little research available investigating the combination of phytase and 25-OH-D₃ on pork quality. Therefore the objective of this experiment was to examine the effect of phytase and 25-OH-D₃ in low P finisher diets on pig performance, nutrient and mineral digestibility, bone mineralisation and pork quality. It is hypothesised that a combination of phytase and 25-OH-D₃ in a low P finisher diet will increase pig performance and bone mineralisation and will improve pork quality above the supplementation of 25-OH-D3 and phytase individually.

**2 Materials and methods**

All experimental procedures described in this work were approved under University College Dublin animal research ethics committee (AREC-13-79-O’Doherty) and conducted under experimental license from the Department of Health in accordance with the cruelty to animal act 1876 and the European communities (amendments of cruelty to animal act, 1876) regulations (1994).

**2.1 Experimental Diets**

The experiment was a 2 × 2 factorial design comprising of four dietary treatments. The dietary treatments were as follows: low P diet (basal) (T1); low P diet + phytase (T2); low P diet + 25-OH-D₃ (T3) and low P diet + phytase + 25-OH-D₃ (T4). The diets were formulated to contain similar levels of digestible energy (13.8 MJ/kg) and standardised ileal digestible lysine (9.5g/kg) (Sauvant, Perez & Tran, 2002). All other amino acids requirements were met relative to lysine according to the ideal protein concept (NRC, 2012). Phytase (Ronozyme®) and the 25-OH-D₃ (Hy-D®) were sourced from DSM, Nutritional Products Limited, UK. Phytase was included at two dietary concentrations, 0 and 500 FYT (phytase units)/kg. A unit is defined as the quantity of enzyme that liberates 1 µmol of inorganic P/min from 1.5 mmol/L of sodium phytase at pH 5.5 at 37°C. The 25-OH-D3 wasadded to the diets at an inclusion rate of 50 µg/kg/feed. All diets were milled on site and offered in meal form. Celite (300 mg/kg) was added to the feed during the milling process in order to measure the coefficient of apparent total tract digestibility (CATTD) of nutrients and minerals using the acid insoluble ash technique (McCarthy, 1977). Feed samples were taken at diet manufacture and throughout the experimental period. The composition and chemical analyses of the dietary treatments are presented in Table 1.

**2.2 Animals and management**

A total of 120 (60 male, 60 female) pigs (Meat line boars × Large White × Landrace sows) (Hermitage, Co. Kilkenny, Ireland) with initial live weight of 60 kg (SD 5.5) were used in this study. The pigs were blocked according to live weight and sex and within each block assigned to one of the four dietary treatments. The pigs were grouped in mixed gender (50:50) groups of ten in 12 pens with a space allowance of 0.9m2 per pig. Before the experiment, the pigs received standard commercial feeding and management. The house was mechanically ventilated and temperature was maintained at 20°C. Each pen had a solid floor lying space with access to slats at the rear of pen. The pens were equipped with single space computerised feeders (Mastleistungsprufung MLP-RAP; Schauer Agrotronic AG, Sursee, Switzerland), as described by Varley et al. (2011), which allowed individual *ad libitum* feeding and daily recording of dietary intake. Briefly, each animal was fitted with a uniquely coded ear tag transponder when an animal entered the feeder, it was recognized by the electronic system (MLP-Manager 1.2; Schauer Maschinenfabrik Ges.m.b.H and CoKG, Prambachkirchen, Austria). When the animal finished feeding and withdrew from the trough, the electronic system recorded the difference between the pre and post visit trough weight and the data were stored in a file with the pen number, the animal’s identification number and the date and the time of entry and exit. The recorded data was used to calculate the individual dietary intake. The pigs were weighed at the beginning of the experiment (day 0) and every two weeks up to slaughter (day 55).

**2.3 Slaughter procedure**

The pigs were slaughtered at a nearby commercial abattoir, Rosderra Irish Meats, Edenderry, Co. Offaly, Ireland, at the end of the 55 day feeding period with an average live weight of 108.4 kg (SD 8.73 kg). The pigs were stunned using carbon dioxide and humanely slaughtered. A blood sample for serum analysis was taken immediately after slaughter via the jugular vein using lithium/heparin vacutainers (BD Plymouth, UK). The blood was stored over night at 4°C and centrifuged at 4720 g for 20 min at 4°C (40R centrifuge, Thermo Fisher Scientific, Ireland) after which the serum layer was subsequently removed from the blood cell layer and stored in 1.5 mL tubes at ‒20°C until required for further P and Ca analysis. The back-fat thickness was measured using the Hennessy grading probe (Hennessy and Chong, Auckland, New Zealand) as described by Egan et al. (2015). The lean meat content was estimated, using the following equations:

Estimate lean meat content (g/kg) = 543.1 – 7.86x + 2.66y (Eq. 1)

where x is fat depth (mm) and y is muscle depth (mm).

Further carcass data were determined using the following equations:

Carcass weight (kg) = hot carcass weight × 0.98 (Eq. 2)

Kill out proportion (%) = carcass weight / body weight (Eq. 3)

Following overnight chilling of the carcasses at 4°C, the *Longissimus thoracis* (LT) muscle was excised from twelve randomly selected pigs per treatment (2 males and 2 females from 3 pens), and subdivided into eight boneless pork steaks per pig (approximately 1.5 cm in thickness). Five steaks were placed in vacuum pack pouches (200 × 300 mm, Mc Donnells Ltd, Dublin, Ireland) and flushed with 80% O2: 20% CO2 (modified atmosphere packs, MAP technology) as described by Rajauria, Draper, Mcdonnell, & O'Doherty (2016) using a single chamber vacuum-sealing unit (Webomatic vacuum packaging system, C10 H with a Km100-3M gas mixer, Germany) for colour analysis. The LT steaks in MAP were stored for up to 14 days at 4°C for colour analysis. The gas atmosphere (oxygen and carbon dioxide) in the MAP was checked using a handheld gas analyser (Checkpoint handheld gas analyser, PBI Dansensor, Denmark). The remaining LT samples were vacuum packed using a single chamber vacuum-sealing unit and were stored at ‒20 ºC until required for vitamin D analysis, water holding capacity and Warner Bratzler shear force (WBSF) analysis. After slaughter, the right front foot of eight pigs per treatment were cleaned of all skin, muscle and connective tissue to remove the third and fourth metacarpals. Following removal, the metacarpals were again cleaned of any remaining flesh. These bones were subsequently used for the assessment of bone ash, P, and Ca levels and bone density. All metacarpals collected were individually stored at –20 °C to prevent desiccation until analysis.

2.4 Laboratory analysis of samples

Feed and faecal samples were analysed for DM, ash, nitrogen (N), ether extract (EE), gross energy (GE), Ca and P content. Following collection, faecal samples were dried at 55°C for 72 h. The feed and dried faecal samples were milled through a 1 mm screen (Christy and Norris Hammer Mill, Chelmsford, England). Crude ash content was determined after ignition of a weighed sample in a muffle furnace (Nabertherm, Bremen, Germany) at 550°C for 6 h. The GE content was determined using an adiabatic bomb calorimeter (Parr Instruments, IL USA). The N content of the feed and faeces was determined as N × 6.25 using the LECO FP 528 instrument (LECO Instruments, UK) (AOAC.990.03, 2005). The EE concentration of the diets was determined using light petroleum ether and Soxtec instrumentation (Tecator, Sweden). The concentration of P in feed and faecal samples was determined spectrophotometrically using the method of Cavell. (1955). A series of concentrations of P standards ranging from 10 to 50 ppm were used for a calibration curve and digested samples diluted to fit this curve. The Ca concentration of feed and faeces samples was determined using an atomic absorption spectrophotometer (Varian '50,' Varian, Santa Clara, CA, USA) using the method of Ramakrishna, West & Robinson, (1968). All samples were measured in duplicate.

Bone samples were analysed for density, ash, Ca and P content. Bone density was determined according to the method of Vigors, Sweeney, O’Shea, Browne & O’Doherty (2014). Briefly bones were firstly weighed in air and then weighed submerged in distilled water using a weigh-below hook facility (Scout Pro balance 200g × 0.01 g, Ohaus Limited). Bone volume was calculated according to Giancoli, Corey & Mullaney, (1998). The bone samples were dried at 100°C for 16 h to calculate DM content and ashed at 650°C in a muffle furnace. The ash was digested in aqua regia (HCl-HNO₃) and analysed for P and Ca content as previously described for the feed and faecal samples.

2.5 Colour measurement

The surface colour of the LT steaks was measured using a colorimeter (CR-400 handheld Chroma meter, Konica Minolta, Co, Oska, Japan) as described by Rajauria et al. (2016). Briefly the Chroma meter was calibrated on the CIE LAB colour system using a CR-A43 calibration plate (Dc: L\* = 97.79, a\*= -0.11, b\*= 2.69). The ‘L\*’ value represents lightness while ‘a\*’ and ‘b\*’ values represent redness and yellowness, respectively. Chroma (C) and hue angle (H\*) were calculated according to Eq. 4 and 5, respectively. The LT samples were stored at 4°C, colour measurements of the steaks were recorded on storage days 0, 4, 7, 11 and 14. These values were recorded in triplicate (averaged from three locations) from each side of the cut of LT.

(Eq. 4)

(Eq. 5)

**2.6 Warner Bratzler shear force and cook loss**

Warner Bratzler shear force analysis was carried out according to Wheeler, Shackelford & Koohmaraie, (1996) with some modifications. Briefly, LT samples were de-frosted overnight at 4°C, trimmed of external fat, weighed and cooked in open vacuum bags in a circulating water bath (Grant Instruments Ltd., Barrington, Cambridge CB2 IBR, UK) set at 72°C, until an internal temperature of 70°C was achieved. Internal temperature was monitored by placing a thermocouple in the geometric centre of each steak, four steaks were cooked per water bath to ensure water circulation was consistent around all samples. All juices was poured out of bag immediately after removing from water bath. The LT steaks were cooled sufficiently, dabbed gently with tissue paper to absorb excess moisture and weights were recorded. The % cook loss was then determined by the following equation:

Cook loss = (Eq. 6)

where X = raw weight of LT and Y = cooked weight of LT.

The LT steaks were allowed to cool fully, placed in storage bags (to prevent dehydration) and were stored at 4°C overnight. Coring was carried out on chilled samples after 24 h, eight (1.25 cm diameter) cores parallel to longitudinal orientation of fibres were collected from each sample and was sheared using an Instron universal testing machine (Model no. 5543, Instron Europe, High Wycombe, Bucks, UK) equipped with a Warner Bratzler shearing device. Cores were sheared at a crosshead speed of 200 - 250 mm/min. Cores that were not uniform in diameter or containing obvious connective tissue defects were discarded. For analysis of the data, Instron Series IX Automated Materials Testing System software for Windows (Instron Corporation, Bucks, UK) was employed.

**2.7 Water holding capacity**

Water holding capacity was carried out according to Honikel. (1998). Briefly, samples were weighed and suspended using a hook and places in a polythene bag, which was tied to prevent dehydration. Samples were hung freely inside the bag to ensure exudate did not remain in contact with the meat. The bag was hung freely for 48 h at 4°C. LT steaks were removed from the bag, gently blotted dry using tissue paper and reweighed. Water holding capacity was calculated using the following equation:

Water holding capacity = (Eq. 7)

where W1 = initial weight and W2 = weight after 48 h.

**2.8 Statistical analysis**

The data was checked for normality and homogeneity of variance by histograms, qq plots, and formal statistical tests as part of the UNIVARIATE procedure of SAS (SAS, 2006). The growth performance was analysed by repeated measures analysis using PROC MIXED procedure of SAS (Littell et al., 1996). The model included the fixed effects of phytase, vitamin D, time, sex and the associated two and three way interactions while the random effect was pen and animal within pen. Initial body weight was used as a covariate for growth performance data. The data on carcass characteristics, shear force, cook loss and water holding capacity of the meat was analysed using PROC MIXED procedure of SAS. The model included fixed effect of phytase, vitamin D and sex and the random effects included pen and animal within pen. The colour variations was analysed by repeated measures analysis using the PROC MIXED procedure of SAS (Littell, Milliken, Stroup & Wolfinger 1996). The statistical model included the fixed effects of phytase, vitamin D, time of sampling and the associated two and three way interactions while the random effect was pen and animal with pen. The mean values were considered to be significantly different when *P <* 0.05 and considered a numerical tendency when *P <* 0.10. Least square means are reported with pooled standard errors.

**3 Results**

**3.1 Performance and carcass characteristics**

The effect of phytase and 25-OH-D₃ inclusion on the growth performance and carcass characteristics of pigs is presented in Table 2 and 3. There was no phytase × 25-OH-D₃ interaction or phytase × 25-OH-D₃ × time interaction on performance characteristics (*P >* 0.05). The pigs offered phytase had a lower daily feed intake (ADFI) (2.45 kg *v.* 2.59 kg; *P <* 0.05) and lower feed conversion ratio (FCR) (2.74 kg/kg *v.* 2.85 kg/kg; *P <* 0.05) compared to the non phytase fed pigs. There was no effect (*P >* 0.05) of phytase or 25-OH-D₃ inclusion on average daily gain (ADG), final body weight, carcass weight, kill-out percentage, back-fat depth, muscle depth or lean meat percentage. Male pigs had a higher (*P <* 0.05) ADG and FCR compared to female pigs.

**3.2 Coefficient of total tract apparent digestibility of nutrients**

The effect of phytase and 25-OH-D₃ inclusion on the CATTD of nutrients and minerals is presented in Table 4. There was no phytase × 25-OH-D₃ interaction (*P >* 0.05) for CATTD of nutrients and minerals. There was no effect (*P >* 0.05) of phytase or 25-OH-D₃ on the CATTD of dry matter, organic matter and gross energy. Pigs offered the phytase diets had an increased CATTD of ash (42.98 *v*. 34.51; SEM 1.351; *P <* 0.01), P (54.45 *v.* 41.24; SEM 2.367; *P <* 0.01) and Ca (40.21 *v.* 26.37; SEM 2.677; *P <* 0.01) compared to pigs offered non-phytase diets. Pigs offered the 25-OH-D₃ supplemented diets had increased CATTD of N (74.03 *v.* 69.89; SEM 1.186; *P <* 0.05), ash (41.33 *v.* 36.17; SEM 1.351; *P <* 0.01) and P (51.41 v. 44.28; SEM 2.267; *P <* 0.01) compared to pigs offered the non-25-OH-D₃ diets.

**3.3 Bone analysis and serum phosphorus and calcium concentration**

The effect of phytase and 25-OH-D₃ inclusion on bone parameters are presented in Table 5. There was no interaction between phytase × 25-OH-D₃ (*P >* 0.05) on bone parameters including, DM, ash, Ca and P content and bone density. Pigs offered the phytase diets had an increased (*P <* 0.05) bone DM, ash, Ca, P and density compared to the pigs offered the non-phytase diets. There was no effect of 25-OH-D₃ on bone parameters. Additionally, there was no phytase, 25-OH-D3 or phytase × 25-OH-D3 interaction (*P >* 0.05) observed for serum P and Ca concentrations.

**3.4 Pork quality**

The effect of phytase and 25-OH-D₃ inclusion on pork quality is presented in Table 6. There was a significant interaction between phytase and 25-OH-D₃ on cook loss % (*P <* 0.05). Muscle from pigs offered 25-OH-D3 hadincreased cook loss % compared to pigs offered the basal diet, however, there was no effect on cook loss % values when phytase and 25-OH-D3 were offered in combination compared to the phytase only diet. There was no interaction between phytase × 25-OH-D₃ (*P >* 0.05) on WBSF values or drip loss (*P >* 0.05). Muscles from pigs offered the 25-OH-D₃ diets had increased WBSF values compared to the pigs offered the non- 25-OH-D₃ diets. There was no effects of phytase or 25-OH-D₃ on pork drip loss.

**3.5 Colour measurement of LT muscle**

The value of colour parameters L\*, a\* and b\* of pork LT samples as a result of the inclusion of phytase and 25-OH-D₃ supplementation is presented in Table 7. There was no phytase × 25-OH-D₃ interactions (*P >* 0.05) for L\*, a\* or b\* values. Muscle from pigs supplemented with 25-OH-D₃ diets exhibited lower (*P <* 0.05) L\* values and higher (*P <* 0.05) red (a\*) values compared to pigs offered the diets without 25-OH-D₃ supplementation. Muscle from pigs supplemented with the 25-OH-D₃ exhibited higher (*P <* 0.05) yellowness (b\*) and Chroma compared to pigs without 25-OH-D₃ supplementation. Muscle from pigs supplemented with phytase had higher (*P <* 0.05) yellowness and Chroma values compared to non-phytase supplemented diets. There was no effect of dietary treatment (*P >* 0.05) on muscle hue angle. The L\* , b\*, Chroma and hue angle values increased in fresh pork samples in all treatment groups (*P <* 0.001) over the 14 day storage period, while the a\* values increased (*P <*0.05) up to storage day 7 and then the values decreased.

**4 Discussion**

Phytase is extensively used in modern swine production to improve P and Ca digestibility and retention (Dersjant-Li et al., 2015) while vitamin D3 supplementation can also increase the utilization of phytate bound P and Ca (Qian et al., 1997). The hypothesis of the current experiment is that supplementation of a combination of phytase and 25-OH-D₃ in a low P finisher pig diet would improve performance, skeletal bone mineralisation and pork quality. However, in the present study there was no phytase × 25-OH-D3 interaction observed for pig performance or skeletal bone mineralisation, and there was also no advantage of offering the combination on pork quality.

The potential of the phytase enzyme to improve the CATTD of P and Ca is well established (Brana, Ellis, Castaneda, Sands & Baker 2006; Selle & Ravindran, 2008). In the current experiment, phytase supplementation increased the CATTD of ash, P and Ca and improved FCR of the pigs. This can be attributed to increased mineral availability as phytase had no effect on nutrient digestibility in the current study. Similarly, Selle, Cadogan & Bryden (2003) reported a high correlation (r = 0.92) between improvements in feed efficiency and phytase supplementation.

The supplementation of 25-OH-D3 increased the CATTD of N, ash and P. Previous work demonstrated that the supplementation of vitamin D3 to broilers enhanced the enterocytes of the small intestine to transport P into the plasma compartment, resulting in an increase in P absorption and retention in the body (Edwards, 1993; Khan, Shahid, Mian, Sarder & Anjum, 2010). The mechanism on how vitamin D increases the CATTD of N is not fully understood. The inclusion of 25-OH-D3, may prevent the formation of protein-phytate complexes within the gut, rendering the protein more susceptible to breakdown by pepsin in the stomach, similar to phytase enzyme (Kies, De Jonge, Kemme & Jongbloed 2006). It is also possible that the 25-OH-D3 molecule may release phytate bound protein, similar to the actions of phytase (Mohammed, Gibney & Taylor 1991), however this warrants further investigation.

Serum Ca and P are generally not sensitive enough to detect problems with mineral imbalances due to the efficient homeostatic mechanism of Ca and P in the body (O'Doherty et al., 2010). Data from the present study indicates no significant difference in serum Ca or P concentrations, however bone parameters including bone ash, Ca, P and density are more reliable indicators of mineral balance in the body (Prentice, 2003; O'Doherty et al., 2010). In the present study there was improvements in bone parameters due to phytase supplementation which can be attributed to the improvement in the CATTD of ash, P and Ca when pigs were offered phytase diets. Phytase inclusion increased bone density and mineral content which is concurrent with previous research carried out by Harper, Kornegay & Schell (1997), however, there was no additional benefit of adding both 25-OH-D3 and phytase in the diet on bone characteristics. Additionally, the supplementation of 25-OH-D3 showed no benefit on bone parameters and this agrees with previous studies investigating the effects of 25-OH-D3 supplementation on bone mineralisation in sows, weaner and grower pigs (Flohr et al., 2014a; Flohr et al., 2014b; Regassa, Adhikari, Nyachoti & Kim 2015; von Rosenberg et al., 2016).

In the present study, muscle from pigs offered 25-OH-D3 had an increase in cook loss over the basal diet; however there was no effect on cook loss when phytase was included in the diet. The reasoning for the increased cook loss with 25-OH-D3 supplementation is unclear. This increase in cook loss maybe due to an altered muscle fibre histology, which may result in thermal denaturation of meat proteins and increased fluid loss. An increased cook loss is associated with greater fluid loss from the muscle which may increase the number of fibres per unit of cross section and in turn may lead to tougher meat (Van Oeckel, Warnants & Boucqué 1999), which explains the higher WBSF values when pigs were supplemented with 25-OH-D₃. A positive correlation (R = 0.43) between fluid loss and shear force of meat samples has been previously established (De Smet, Bloemen, Van De Voorde, Spincemaille & Berckmans 1998). However, while the WBSF values increased with the supplementation of 25-OH-D3 the value indicated a tenderness level within the range acceptable for consumer consumption (Van Oeckel et al., 1999).

The pork colour change due to vitamin D supplementation may be attributed to the potential of vitamin D3 to shift muscle metabolism to be more oxidative and thereby decreasing the rate and extent of pH decline, thus improving colour (Wilborn, Kerth, Owsley, Jones & Frobish 2004). This may have contributed to lower lightness, increased redness and saturation (Chroma) values. The increased redness may be also due to the possible antioxidative capacity that has been previously linked to vitamin D3 (Lahucky et al., 2007; Hansen, Frylinck & Strydom 2012). The increase in Chroma (derived from a\* and b\* values) with phytase supplementation reflects a higher yellowness (decreased blueness) score since redness was unaffected by phytase supplementation. The mechanism to explain the increased yellowness with phytase supplementation (and indeed with 25-OH-D3 supplementation) is unclear although it could reflect an increase in oxymyoglobin/deoxymyoglobin ratio close to the meat surface. Luciano, Monahan, Vasta, Pennisi, Bella & Priolo (2009) reported H\* as an appropriate evaluation of meat colour deterioration with storage giving a more realistic perspective on meat browning than single colour coordinates. Regardless of dietary treatment in the present study, H\* values increased over the storage period and there was no effect of dietary treatments.

**5 Conclusion**

The present study demonstrates that there was no advantage of offering a combination of phytase and 25-OH-D3 on pig performance, bone parameters or pork quality compared to offering these feed additives individually. The supplementation of phytase improved FCR and increased the bone DM, ash, Ca, P and density compared to the non-phytase diets. Pork from pigs supplemented with 25-OH-D3 had lower L\* values. The supplementation of phytase and 25-OH-D3 individually resulted in higher C values, however no dietary effect was observed for muscle H\* values.

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**Table 1**

Composition and chemical analysis of the basal diet offered to pigs over the experimental period (g/kg, unless otherwise stated)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Dietary treatments | | | |
| Phytase | No | Yes | No | Yes |
| 25-OH-D3 | No | No | Yes | Yes |
| *Ingredients* |  |  |  |  |
| Wheat | 382.6 | 382.6 | 382.6 | 382.6 |
| Barley | 250.0 | 250.0 | 250.0 | 250.0 |
| Soya bean | 170.0 | 170.0 | 170.0 | 170.0 |
| Maize | 150.0 | 150.0 | 150.0 | 150.0 |
| Soya oil | 18.0 | 18.0 | 18.0 | 18.0 |
| Salt | 5.0 | 5.0 | 5.0 | 5.0 |
| Monocalcuim phosphate | 6.6 | 6.6 | 6.6 | 6.6 |
| Limestone | 12.5 | 12.5 | 12.5 | 12.5 |
| Lysine HCl | 2.30 | 2.30 | 2.30 | 2.30 |
| L-threonine | 0.50 | 0.50 | 0.50 | 0.50 |
| Vitamins and mineral premix† | 2.50 | 2.50 | 2.50 | 2.50 |
| Phytase (FYT/kg) | 0 | 500 | 0 | 500 |
| 25-OH-D₃ (µg/kg) | 0 | 0 | 50 | 50 |
| *Chemical analysis (g/kg)* |  |  |  |  |
| Dry matter | 886.4 | 886.3 | 886.5 | 885.7 |
| Crude protein (N × 6.25) | 168.9 | 168.5 | 168.8 | 169.0 |
| Ash | 44.7 | 43.6 | 43.4 | 43.6 |
| Gross energy (MJ/kg) | 15.8 | 15.9 | 16.0 | 15.8 |
| Ether extract | 24.5 | 24.5 | 23.5 | 23.1 |
| Lysine‡ | 10.5 | 10.5 | 10.5 | 10.5 |
| Methionine and cysteine‡ | 6.30 | 6.30 | 6.30 | 6.30 |
| Threonine‡ | 7.20 | 7.20 | 7.20 | 7.20 |
| Tryptophan‡ | 1.90 | 1.90 | 1.90 | 1.90 |
| Calcium | 5.97 | 6.01 | 5.96 | 6.04 |
| Phosphorous | 4.81 | 4.85 | 4.84 | 4.77 |

† The premix provided vitamins and minerals (per kg diet) as follows: 0.01g/kg of retinol acetate, 0.16 g/kg of alpha tocopherol acetate, 0.007 g/kg of menadione, 0.00125 g/kg of thiamine mononitrate, 0.005 g/kg of riboflavin, 0.0025 g/kg of pyridoxine HCl, 0.003 g/kg of cyanocobalamin,0.0229 g/kg of nicotinamide, 0.0138 g/kg of calcium-D-pantohenate, 0.06 g/kg of copper as copper sulphate, 0.4167 g/kg of iron as iron sulphate, 0.0806 g/kg of manganese as manganese oxide, 0.0032 g/kg of iodine as calcium iodate, 0.1389 g/kg of zinc as zinc oxide, 0.0056 g/kg selenium, 1.24 g/kg of calcium.

‡ Calculated for tabulated nutritional composition (Sauvant et al., 2004).

**Table 2**

Effect of phytase and 25-OH-D3 on animal growth performance (Least square means ± SEM)

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Treatment | | | |  | Sex | |  | Significance\* | | | | |
| Phytase | No | Yes | No | Yes | SEM1 | F | M | SEM | Phytase | 25-OH-D3 | Phytase ×  25-OH-D3 | Time | Sex |
| 25-OH-D3 | No | No | Yes | Yes |
| ADG (kg/d) | 0.93 | 0.92 | 0.92 | 0.89 | 0.021 | 0.89 | 0.94 | 0.015 | 0.484 | 0.396 | 0.592 | 0.737 | 0.009 |
| ADFI (kg/d) | 2.56 | 2.45 | 2.62 | 2.42 | 0.056 | 2.51 | 2.54 | 0.041 | 0.018 | 0.978 | 0.238 | 0.001 | 0.662 |
| FCR (kg/kg) | 2.81 | 2.74 | 2.89 | 2.74 | 0.061 | 2.87 | 2.71 | 0.042 | 0.050 | 0.539 | 0.442 | 0.001 | 0.009 |
| Final BW (kg) | 108.7 | 108.0 | 108.9 | 106.7 | 1.14 | 107.7 | 108.0 | 0.56 | 0.138 | 0.505 | 0.5692 | 0.001 | 0.889 |

SEM = Standard error of the mean.

ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio; BW, body weight; F, female; M, male.

\*There was no Phytase × 25-OH-D₃ × Time (*P >* 0.05) interaction.

**Table 3**

Effect of phytase and 25-OH-D3 on carcass characteristics. (Least square means ± SEM)

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Treatment | | | |  | Sex | |  | Significance\* | | | |
| Phytase | No | Yes | No | Yes | SEM | F | M | SEM | Phytase | 25-OH-D3 | Phytase × 25-OH-D3 | Sex |
| 25-OH-D3 | No | No | Yes | Yes |
| Carcass weight (kg) | 80.8 | 79.5 | 81.3 | 79.6 | 1.612 | 80.6 | 80.1 | 1.74 | 0.138 | 0.505 | 0.569 | 0.778 |
| Kill out % | 74.6 | 74.5 | 74.7 | 74.3 | 0.607 | 74.9 | 74.2 | 0.438 | 0.347 | 0.844 | 0.893 | 0259 |
| Back-fat depth (mm) | 13.9 | 13.5 | 14.5 | 14.7 | 0.673 | 14.1 | 14.3 | 0.472 | 0.629 | 0.929 | 0.746 | 0.756 |
| Muscle depth (mm) | 55.9 | 55.4 | 56.4 | 55.9 | 1.481 | 56.1 | 55.8 | 1.072 | 0.945 | 0.186 | 0.618 | 0.823 |
| Lean meat % | 56.8 | 55.8 | 56.0 | 55.5 | 0.572 | 56.3 | 55.6 | 0.402 | 0.731 | 0.727 | 0.984 | 0.237 |

SEM = Standard error of the mean.

F = female; M = male.

\*There was no phytase × 25-OH-D₃ × Time (*P >* 0.05) interaction.

**Table 4**

Effect of phytase and 25-OH-D3 on the coefficient of total tract digestibility of dry matter, organic matter, nitrogen, ash, calcium, phosphorus and gross energy (Least square means ± SEM)

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Treatment | | | |  | Significance\* | | |
| Phytase | No | Yes | No | Yes | SEM | 25-OH-D₃ | Phytase | 25-OH-D₃ ×  Phytase |
| 25-OH-D₃ | No | No | Yes | Yes |
| *Digestibility coefficients* |  |  |  |  |  |  |  |  |
| Dry matter | 0.77 | 0.79 | 0.79 | 0.79 | 0.009 | 0.249 | 0.321 | 0.281 |
| Organic matter | 0.79 | 0.80 | 0.81 | 0.80 | 0.009 | 0.349 | 0.547 | 0.266 |
| Nitrogen | 0.68 | 0.73 | 0.74 | 0.74 | 0.002 | 0.027 | 0.109 | 0.164 |
| Gross energy | 0.75 | 0.77 | 0.77 | 0.77 | 0.112 | 0.447 | 0.471 | 0,322 |
| Ash | 0.32 | 0.40 | 0.37 | 0.46 | 0.019 | 0.011 | 0.001 | 0.997 |
| P | 0.38 | 0.51 | 0.45 | 0.58 | 0.036 | 0.034 | 0.001 | 0.994 |
| Ca | 0.24 | 0.39 | 0.29 | 0.43 | 0.040 | 0.185 | 0.001 | 0.885 |

SEM = Standard error of the mean.

P = Phosphorus and Ca = calcium.

\*There was no effect of sex (*P >* 0.05).

**Table 5**

Effect of phytase and 25-OH-D3 on bone parameters in the third metacarpal of the right front foot of pigs at slaughter (day 55) and serum analysis. (Least square means ± SEM)

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Treatments | | | |  | Significance\* | | |
| Phytase | No | Yes | No | Yes | SEM | 25-OH-D₃ | Phytase | 25-OH-D₃ ×  Phytase |
| 25-OH-D₃ | No | No | Yes | Yes |
| *Bone analysis (g/kg)* |  |  |  |  |  |  |  |  |
| Dry matter | 713.6 | 752.8 | 730.9 | 737.7 | 9.882 | 0.912 | 0.028 | 0.115 |
| Ash | 368.6 | 442.9 | 377.4 | 399.9 | 13.951 | 0.233 | 0.002 | 0.075 |
| Ca | 133.9 | 162.6 | 136.7 | 143.9 | 5.229 | 0.144 | 0.002 | 0.061 |
| P | 66.9 | 80.3 | 65.4 | 70.8 | 3.073 | 0.083 | 0.005 | 0.209 |
| Density† | 1.34 | 1.48 | 1.33 | 1.37 | 0.435 | 0.184 | 0.052 | 0.242 |
| *Serum analysis* |  |  |  |  |  |  |  |  |
| Serum P (nmol/L) | 4.05 | 4.25 | 4.27 | 3.91 | 0.168 | 0.727 | 0.620 | 0.112 |
| Serum Ca (nmol/L) | 2.85 | 2.85 | 2.79 | 2.79 | 0.037 | 0.163 | 0.869 | 0.869 |

SEM = Standard error of the mean.

†Density was calculated according to the method of Giancoli et al. (1998).

P = phosphorus and Ca = calcium.

\*There was no (*P >* 0.05) effect of sex.

**Table 6**

Effect of phytase and 25-OH-D3 on pork quality parameters including drip loss %, cook loss % and Warner Bratzler shear force value on d 0. (Least square means ± SEM)

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Treatments | | | |  | Significance\* | | |
| Phytase | No | Yes | No | Yes | SEM | 25-OH-D₃ | Phytase | 25-OH-D₃ ×  Phytase |
| 25-OH-D₃ | No | No | Yes | Yes |
| Drip Loss (%) | 11.7 | 10.3 | 11.4 | 11.1 | 0.672 | 0.782 | 0.216 | 0.415 |
| Cook Loss (%) | 27.5 | 28.7 | 30.6 | 27.6 | 1.020 | 0.342 | 0.356 | 0.047 |
| WBSF (N) | 40.7 | 42.4 | 44.9 | 43.4 | 1.160 | 0.037 | 0.927 | 0.189 |

SEM = Standard error of the mean.

\*There was no (*P >* 0.05) effect of sex.

**Table 7**

Effect of phytase and 25-OH-D3 on surface colorimeter values ‘L\*’ lightness, ‘a\*’ redness, ‘b\*’ yellowness, ‘C’ Chroma and ‘H\*’ Hue angle of fresh *Longissimus thoracis* (LT) stored in modified atmosphere packs (80% O2: 20% CO2) for up to 14 days at 4°C.(Least square means ± SEM)

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Phytase | | |  | 25-OH-D₃ | | |  | Time | | | | | |  | Significance† | | |
|  | Yes | No | SEM |  | Yes | No | SEM |  | 0 | 4 | 7 | 11 | 14 | SEM |  | Phytase | 25-OH-D₃ | Time |
| L\* | 53.3 | 52.9 | 0.198 |  | 52.8 | 53.4 | 0.198 |  | 49.7 | 52.9 | 53.5 | 54.3 | 55.1 | 0.313 |  | 0.181 | 0.050 | 0.001 |
| a\* | 4.7 | 4.7 | 0.103 |  | 4.8 | 4.5 | 0.103 |  | 4.46 | 5.20 | 5.19 | 4.76 | 3.87 | 0.163 |  | 0.994 | 0.009 | 0.001 |
| b\* | 4.1 | 3.6 | 0.095 |  | 4.1 | 3.6 | 0.095 |  | 1.15 | 2.95 | 4.22 | 5.03 | 5.94 | 0.149 |  | 0.001 | 0.009 | 0.001 |
| C | 6.5 | 6.2 | 0.081 |  | 6.6 | 6.1 | 0.081 |  | 4.66 | 6.02 | 6.77 | 7.02 | 7.18 | 0.129 |  | 0.024 | 0.001 | 0.001 |
| H\* | 38.9 | 36.5 | 1.01 |  | 37.9 | 37.5 | 1.01 |  | 16.7 | 29.3 | 38.8 | 46.8 | 57.0 | 1.556 |  | 0.092 | 0.735 | 0.001 |

L\*= lightness, a\*= redness, b\*= yellowness, C= Chroma, H\*= Hue angle.

SEM = Standard error of the mean.

†There was no phytase × 25-OH-D₃ interaction (*P >* 0.05) or sex effect (*P >* 0.05).