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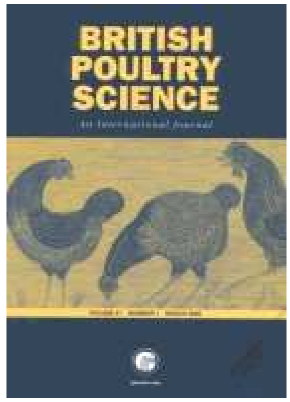

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Characterisation of European varieties of triticale with special emphasis on the ability of plant phytase to improve phytate phosphorus availability to chickens

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Abstract 1. A total of 30 varieties and selection lines of triticale grown under similar conditions were characterised. Thousand grain weight, specific weight, Hagberg falling number and N were 50.2 ± 5.0 g, 72.4 ± 2.1 kg/hl, 96 ± 48 s and 16.1 ± 0.11 g/kg, respectively.

2. Mean phosphorus (P) concentration was 2.86 ± 0.31 g/kg, of which 77% was of phytic origin. Mean phytase activity was 1018 ± 319 PU/kg. A genotypic effect on phytase activity was detected amongst 5 varieties studied out of 30. Potential and real applied viscosities were positively correlated and mean values were 3.53 ± 0.66 and 2.15 ± 0.31 ml/g, respectively.

3. The efficacy of plant phytase in improving P availability was assessed in chickens up to 3 weeks of age. Growth performance and bone ash concentration were compared in birds given either a maize (450 g/kg) and soybean meal (230 g/kg) phosphorus deficient diet containing 3.5 g P/kg, this basal diet supplemented with 1 or 2 g P/kg as monocalcium phosphate (MCP) or triticale (450 g/kg) and soybean meal (230 g/kg) diets containing 3.2 to 3.8 g P/kg with no MCP. To achieve graded levels of phytase activity, 4 varieties of triticale, intact or in which phytase was denaturated by heat treatment, were used. Estimated metabolisable energy, protein, amino acids and calcium concentrations were similar in all diets.

4. Phytase activity in the triticale-based diets ranged between 135 and 1390 PU/kg. Growth performance and bone ash were responsive to plant phytase and to MCP. Non-linear models of these responses were adjusted with the best fit for bone ash parameters. 250, 500 and 1000 PU of plant phytase were estimated to be equivalent to 0.46, 0.67 and 0.81 g P as MCP, respectively.

INTRODUCTION

Triticale is a hybrid of wheat and rye that has been proposed as an alternative cereal in animal feeding because of its potential combination of wheat feeding characteristics and rye winter hardiness and disease resistance (Gatel *et al*., 1985; Vieira *et al*., 1995). Several grain constituents play a role in optimum utilisation of this cereal by poultry. Among them, both soluble non-starch-polysaccharides (NSP), mainly arabinoxylans present in the albumen, and available phosphorus (P) are of great impact with regard to the nutritive value of the cereal and to the alimentary strategies to control environmental pollution problems (Carré *et al.*, 1994; Barrier-Guillot *et al.*, 1996b) and are worth consideration in varietal selection programmes. Improvements in nutritional value of triticale for broilers will increase the economical interest in replacing wheat by triticale in broilers (Korver *et al*., 2004). Protein concentation and its amino acid profile as well as metabolisable energy are key contributors to feeding value. High contents of arabinoxylans should be avoided, because this might increase feed viscosity, reduce digestibility of various components and induce over-consumption of water by birds, which results in aqueous excreta (Carré *et al.*, 2002). In contrast, high P availability reduces the requirement for supplementing diets with mineral P leading to lower excretion of this element by the animals.

The effect of arabinoxylans on viscosity can be assessed by means of real applied viscosity (RAV) and potential applied viscosity (PAV) which refer to viscosity measured when endogenous xylanases are allowed to act and are inactivated, respectively (Carré *et al.*, 1994; Carré, 2002). In wheat, these parameters are factors of variation in metabolisable energy (Carré *et al.*, 2002). Bouguennec *et al.* (2000) reported PAV values from 1.6 to 5.1 for 49 varieties of triticale from the official French catalogue and highlighted that PAV is mainly under genetic control, even if it may also be affected by environmental conditions. Similarly in wheat, RAV and PAV are dependent on the genotype, although RAV, which depends on an enzymatic activity, is affected by an environment x genotype interaction (Oury *et al.*, 1998).

In wheat, variability in P availability for poultry is mainly related to phytase activity but not to phytic P concentration (Barrier-Guillot *et al.*, 1996b). Moreover, phytase activity displays a significant genotypic effect in wheat, although it is also influenced by a genotype x environment interaction (Barrier-Guillot *et al.*, 1996a; Oury *et al.*, 1998). Triticale has been reported to display a phytase activity intermediate between those of wheat and rye (770, 460 and 5350 phytase units (PU)/kg, respectively according to INRA-AFZ, 2004) but the available studies (Eeckhout and De Paepe, 1994; Nys *et al.,* 1996; Skiba *et al.,* 2004) do not provide information about the actual genotypic variability. Besides, the effectiveness of plant phytase to improve P availability in chickens has mainly been studied in wheat (Barrier-Guillot *et al.*, 1996b; *Potkansky*, 2000; Paik, 2003; Juanpere *et al.*, 2004), with little information available for triticale. Almost no information about the potential sparing effect of triticale on the need for mineral P supplementation is available.

The aim of the present study was to evaluate the genotypic variability, in triticale, of several characteristics including viscosity and phytase activity. In addition, the efficacy of plant phytase contained in triticale to reduce the required mineral P supplementation in diets for chickens was evaluated.

MATERIALS AND METHODS

Triticale varieties and lines

A total of 30 varieties and selection lines of triticale originating from different areas in Europe and covering a wide range of genotypic variability were selected. They were grown at the INRA research station in Clermont-Ferrand under a single crop management system adapted to the local conditions. The 30 batches were each collected from a single plot (Table 1). In addition, 5e varieties (DI34-2, Trimaran, Aubrac, Capitale and Calao) were chosen for the wide range in phytase activity they displayed. They were each grown on 4 - 6 plots and were used to assess the genotypic effect on phytase activity. Four out of these 5 latter varieties (DI34-2, Trimaran, Aubrac and Calao) were used to assess response of chickens to graded levels of plant phytase. For this latter study, a mixture in equal proportions of the 4 to 6 available sub-batches of each variety was constituted.

Table 1 near here

Broiler experiment

The experiment was conducted under the animal research guidelines of the French Ministry of Agriculture. From hatching till 5 d of age, 220 male Ross white chicks were placed in plastic-coated cages of two or three birds each and fed on a standard diet covering all nutrient requirements (INRA, 1989). At d 5, chicks were individually weighed after an overnight fast and the 192 chicks closest to the mean weight of 75.7 ± 1.78 g were placed in 96 plastic-coated cages, with two birds of similar initial live weight in each. For the subsequent 16-d experiment, chicks were fed on one of 12 experimental diets (8 cages per diet). The initial room temperature of 32°C was gradually decreased to to 30 and 28°C when the chicks reached the ages of 2 and 14 d, respectively. During the first 2 d, birds were kept under 24 h light a d and 23 h light per d thereafter. Birds had free access to water throughout the experiment. After a 12 to 14-h fast, each chicken was weighed and killed and its right tibiotarsus was collected. Feed consumption was recorded per cage for the 16-d experimental period. Feed conversion ratio (FCR) was calculated as the ratio of feed intake to weight gain over the 16-d experimental period.

Twelve diets were formulated to meet all nutrient requirements of the birds (INRA, 1989), except available P. Feedstuffs were ground in a hammer mill fitted with a 2.5 mm screen prior to incorporation in the diet. Mixed diets were pelleted without steam addition. Damage to plant phytase was not expected, as outlet temperature did not exceed 50°C. The three first diets, with a phytase activity below the detection limit of 50 PU/kg, were based on maize (450 g/kg) and soybean meal (230 g/kg) and contained graded levels of monocalcium phosphate (MCP, 0, 1 and 2 g/kg in diets 1, 2 and 3, respectively) (Table 2). The basal diet without any inorganic phosphorus contained 3,5 g P/kg and was deficient in non-phytic phosphorus compared to the requirement for growing chickens. Diets 4 to 12, did not contain any maize but 450 g/kg of triticale. No MCP was added. In order to achieve graded levels of phytase activity, four different genotypes of triticale (DI34-2, Trimaran, Aubrac and Calao), raw or heat-treated, were introduced in the diets 4 to 7 and 8 to 11, respectively. A mixture of raw (200 g/kg) and heat-treated Calao (250 g/kg) was introduced in diet 12. Heat treatment, which aimed at reducing phytase activity, consisted in two successive heatings in a microwave oven at 600 W for 2 minutes. Measured phytase activity in the raw triticale-based diets and in the heat-treated triticale-based diets was 620 to 1390 PU/kg and 135 to 180 PU/kg, respectively. It was 645 PU/kg in the diet containing the mixture of raw and heated Calao. Maize starch, wheat straw, soy isolate, vegetable oil, calcium carbonate and DL-methionine were used to balance the diets so that estimates of metabolisable energy, protein, lysine, sulphur amino acids, phytic P and Ca concentrations were similar in maize-based diets and in triticale-based diets (INRA, 1989). Moreover, the varieties of triticale were chosen so that dietary RAV remained below 1.40 mlL/g to ensure a limited effect of this parameter on dietary metabolisable energy (Maisonnier *et al.*, 2001). At the end, the 12 diets provided similar and adequate amounts of nutrients for chicks except available P (INRA, 1989).

Table 2 near here

Sampling and analyses

Analyses were performed in duplicate or in triplicate. Thousand grain weight (TGW) was assessed by passing 200 g of fresh grain through an automated Decca seed counter and adjusting weights to 15% moisture content. Specific weight (SW) was measured by means of a Nilema apparatus (Tripette et Renaud, France). The Hagberg falling number (HFN) was assessed by means of a Falling Number apparatus type 1400 (Falling Number AB, Sweden) after grinding with a Falling Number mill type KT120 (Falling Number AB, Sweden). Samples of triticale and diets were ground to pass through a 0.5 mm screen and stored at 4°C prior to the other analyses. N was determined by the Kjeldahl method according to the French standard AFNOR (NFV 18-100) using a Kjelfoss apparatus (A/S N Foss Electric, Denmark). Dry matter (DM) was measured by drying at 103°C until constant weight. P was analysed by means of the vanadate colorimetric method according to the AFNOR method (NFV 18-106). Phytic P was determined by ion-pair HPLC (Column C_{18}, Hypersyl C 18-5 µm 200 x 2 mm, Interchim) after acidic extraction and anionic exchange purification according to the method developed by Sandberg and Adherrine (1986) and modified by Lehrfeld (1989). Phytase activity (PA) was measured colorimetrically after incubation in a sodium phytate solution (Engelen *et al.*, 1994). One phytase unit (PU) is the amount of enzyme that liberates 1 µmol per minute of inorganic P from 5.1 mmol/l solution of sodium phytate, at pH 5.5 and 37°C. Prior to viscosity determination, extraction (pH = 4.5, temperature = 19-23°C) was performed with or without pre-treatment in hot ethanol: water (80: 20). RAV and PAV refer to viscosity measured when endogenous xylanases were allowed to act and were inactivated by treatment of the sample, respectively. The viscosity data were divided by the viscosity of the buffer, which gave relative viscosities (Vr), transformed into natural logarithm and then divided by the concentration (g/mL) of the plant material in the buffer extraction volume. The results "(Ln(Vr))/(g/ml)" were expressed as ml /g (Carré *et al.*, 1994; Carré, 2002).

Tibiotarsi were pooled per cage. They were cleaned of all soft tissues, defatted (24 hours in ether), dried (105°C for 12 hours) and weighed. Thereafter, they were ashed at 550°C for 14 h in a muffle furnace and weighed. Ash concentration was calculated on a fat-free dry matter (FF DM) basis.

Statistical analyses

Statistical analysis of data was performed by means of the GLM procedure of the Statistical Analysis Systems software package version 8.1 (SAS, 1990). Correlation coefficients (*r*) between physical and analytical characteristics of the 30 batches of triticale were calculated. The effect of the variety on phytase activity was assessed by an analysis of variance using the plot as the experimental unit, followed by a comparison of means. The chickens' responses were analysed using the cage as the experimental unit. Data were first submitted to an analysis of variance with the diet as main factor, followed by a comparison of means. Polynomial regression of the indicators of performance and of bone mineralisation was used to determine the presence of linear and quadratic effects of mineral P added in the diet and of dietary phytase activity. Effects were considered significant when $P<0.05$.

Non-linear (NLIN procedure of SAS) functions were fitted to the response of performance and bone FF DM and ash to dietary P as MCP and phytase activity, using treatment means. Exponential models were chosen to describe the response to phytase and linear or exponential models were chosen to describe the response to mineral P, according to the results obtained by polynomial regression. The exponential model was chosen because it was extensively used to describe the effect of microbial phytase on P availability (Kornegay *et al.*, 1996; Kornegay, 2001). The model was $Y = a + b\,(1 - e^{-l\,MinP}) + c\,(1 - e^{-k\,Phyt})$ or $Y = a + b\,MinP + c\,(1 - e^{-k\,Phyt})$ with Y = response measurement, MinP = P added as MCP (g/kg diet), Phyt = phytase activity (PU/kg diet).

The coefficient of determination (R^2) of each equation generated was calculated as the square of the correlation coefficient between predicted and observed individual values. An equivalency value of mineral P as MCP (g) for plant phytase (PU) was calculated by setting equal the terms corresponding to mineral P and phytase activity.

RESULTS AND DISCUSSION

Physical and chemical characteristics of the 30 batches of triticale

Physical and chemical characteristics of the 30 batches of triticale are presented in Table 1. Phytase activity and especially HFN displayed the highest degree of variation, with coefficients of variation (CV) of 31 and 49%, respectively. PAV and RAV exhibited a CV of 15-20%. Phytic P displayed a CV of similar order, although with only 11 measurements performed. The other parameters (TGW, N and total P) had lower amplitude of variation (CV 7-10%). This amplitude of variation represents genotypic variability since all the varieties were grown under similar conditions.

Total P concentration in the 30 batches of triticale ranged between 2.06 and 3.57 g/kg with a mean of 2.86 ± 0.31 g/kg. This average P concentration was slightly below the values of 3.7 ± 0.2 and 3.9 ± 0.1 g P/kg previously observed by Eeckhout and De Paepe (1994) and Skiba *et al.* (2004) when analysing 6 and 4 varieties of triticale, respectively. Phytic P was measured in only 11 out of the 30 batches and ranged between 1.50 and 2.62 g/kg, with a mean of 2.18 ± 0.40 g/kg. These values compare with the 2.5 ± 0.2 and 2.4 ± 0.2 g phytic P/kg reported in the two aforementioned studies. As previously observed in wheat (Barrier-Guillot *et al.*, 1996a; Viveros *et al.*, 2000; Kim *et al.*, 2002), P and phytic P were positively correlated (r = 0.60, P<0.05) (Table 3). However, the low value of r hampers a reliable prediction of phytate P from total P. In the current study, 77% of P was, on average, of phytic origin. This is quite a high proportion compared with the 67 and 61% reported by Eeckhout and De Paepe (1994) and Skiba *et al.* (2004), which may be a consequence of the lower total P observed in the current study. P and phytic P concentrations in wheat are influenced by crop management, especially N and P fertilisation (Barrier-Guillot *et al.*, 1996a; Oury *et al.*, 1998). Consequently, the current results may have been influenced by the conditions under which triticale was grown.

Table 3 near here

Phytase activity measured in the 30 batches of triticale ranged between 447 and 1843 PU/kg, with a mean value of 1018 ± 319 PU/kg. As expected, these values are intermediate between the phytase activities reported in wheat and in rye (460 and 5350 PU/kg, according to INRA-AFZ, 2004), although they are closer to those of wheat. These values are below the 1688 ± 227 and 1784 ± 386 PU/kg published by Eeckhout and De Paepe (1994) and Skiba *et al.* (2004), respectively, but compare with the 1190 ± 52 PU/kg measured by Nys *et al.* (1996)

in 5 batches of triticale. These differences may originate from the huge inter-laboratory variability in phytase activity determination mentioned by Tran and Skiba (2005), even when similar analytical methodologies are implemented. Phytase activity determination might be sensitive to the condition under which batches are stored prior to analysis (temperature, duration of storage if the gain is ground) (Nys *et al.*, 1996) and to the way phytase is extracted before analysis of the liberated inorganic phosphates (Greiner and Egli, 2003).

Phytase activity measured in each of the 5 varieties of triticale grown on 4 to 6 plots (Table 4) was 20 to 48% higher than the value previously obtained on the single batch of the same variety (Table 1). Nevertheless, the varieties were similarly ranked in the two sets of data, with a coefficient of correlation of 0.95 (P<0.05, n = 5). The origin of this systematic difference is not clear but it may be ascribable to the interaction between the grinding of the samples before analysis and the duration of their storage (Nys *et al.*, 1996). Phytase activity differed (P<0.01) between varieties. In Calao, Capitale, Aubrac and Trimaran, it exceeded by 112, 79, 41 and 30%, respectively, the value of 1012 PU/ kg in DI34-2. This genotypic effect on phytase activity was previously reported in wheat (Barrier-Guillot *et al.*, 1996a; Kim *et al.*, 2002; Oury *et al.*, 1998; Tran and Skiba, 2005). However, Oury *et al.* (1998) detected a strong genotype x environment interaction in the phytase activity of wheat. This interaction, which may also exist in triticale, was not investigated in the current study. Phytase activity was negatively correlated (P<0.05) with SW and HFN, which is consistent with the fact that low HFN (and possibly SW) might be linked with the stimulation of enzymatic activity in the grain at germination as observed for amylase (Niziolek *et al.*, 1994). As previously reported in wheat (Eeckhout et de Paepe, 1994; Barrier-Guillot *et al.*, 1996a) no relationship between phytase activity and P or phytic P could be detected. In contrast, Viveros *et al.* (2000) found a positive correlation between phytase activity and P in wheat; however, they were not able to predict phytate P or phytase activity reliably from total P.

Table 4 near here

PAV and RAV ranged between 2.35 and 4.65 ml/g and 1.71 and 2.86 ml/g, respectively, with average values of 3.53 ± 0.66 and 2.15 ± 0.31 mL/g, respectively (Table 1). The PAV values are in the range of those reported by Bouguennec *et al*. (2000) in triticale. They are between those observed in rye (PAV = 28 mL/g, Carré *et al.*, 1994) and wheat (PAV = 2.9 mL/g, Oury *et al.*, 1998; Carré *et al.*, 2002), although they are closer to those of wheat. As previously reported (Oury *et al.*, 1998), PAV and RAV were positively correlated (r = 0.43, P<0.05) with each other but not with any other characteristic.

Nitrogen concentration in the 30 varieties of triticale ranged between 14.1 and 18.1 g N/kg (Table 1), corresponding to a mean level of protein of 101 ± 8.8 g/kg with a range of 88 to 113 g/kg. This crude protein level is slightly lower than those observed in 8 Australian cultivars (range 101-135 g/kg; Johnson and Eason, 1988) or that analysed by Vieira *et al.* (1995) (129 g/kg) but is in agreement with levels observed by other authors: 72-110 g/kg (Zacarias *et al*., 1982), 93 g/kg (Proodfoot and Hulan, 1988).

Triticale phosphorus availability in broilers

The microwave treatment applied to triticale was effective in reducing phytase activity, which was reduced after treatment by 85-90%, down to 142, 156, 126, 127 PU/kg in DI34-2, Trimaran, Aubrac and Calao, respectively. Phytase activity measured in diets exceeded the value expected from measurements performed on the batches of triticale, especially at low levels of phytase activity (Table 2). However, phytase activity measured in the batches of triticale and in the diets were highly correlated (r = 0.99, P<0.001, n = 8). RAV in non-heated triticale-based diets was 10 to 23% lower than in heated triticale-based diets, suggesting that heat treatment reduced xylanase activity. However, it remained below 1.40 ml/g, even in heated triticale-based diets. In the current study, heat treatment was considered to have no effect on phytate concentration because phytates present in cereals are very stable to heating (Reddy *et al.*, 1989; Juanpere *et al.*, 2004).

Performance and bone characteristics of birds are presented in Table 5. Five birds fed the maize based diet without MCP (diet 1) died before the end of the experiment, explaining the removal of data from three cages. A high mortality level has previously been observed in chicks fed on maize based diets without supplemented mineral P (Kornegay *et al.*, 1996; Paik, 2003).

Table 5 near here

For all the variables studied, significant differences (P<0.001) among diets were detected. Chicks given the maize-based diets without phosphate and supplemented with 2 g P as MCP displayed the lowest performance and the highest bone characteristics. The linear response of bone ash to graded level of inorganic phosphorus demonstrates that the chicks fed on negative control diet were deficient in phosphorus. Performance and bone characteristics in chickens given the raw triticale-based diets did not differ (P>0.05) from those of chickens given the maize-based diet supplemented with 1 g P as MCP/kg, except for bone ash concentration in the Trimaran-based diet, which was lower. Overall, bone characteristics and final weight of chickens given the heated triticale-based diets were intermediate between those of chickens given the unsupplemented maize-based diet and the raw triticale-based diets. Except for the heated DI-34-2-based diet, feed intake in chicks given the heated triticale-based diets did not differ from that in chicks fed the unsupplemented maize-based diet, while their FCR did not differ from that in chicks fed the raw triticale-based diets. Chickens given the maize-based diet supplemented with 2 g P as MCP/kg ingested 2.1 times more feed than chickens given the maize-based diet without MCP. Feed intake increased linearly (P<0.001) and quadratically (P<0.05 and P<0.001, respectively) with supplemental P supply and with the dietary level of phytase. By the end of the experiment, birds fed on the maize-based diet supplemented with 2 g P as MCP/kg were 2.2 times heavier than birds given the maize-based diet without added P. Final weight increased linearly (P<0.001) and quadratically (P<0.01 and P<0.001, respectively) with P addition and with dietary phytase activity (Figure). FCR decreased linearly and quadratically with added P (*P*<0.01 and *P*<0.05, respectively) and dietary phytase activity (*P*<0.001 and *P*<0.01, respectively). FCR was decreased by 30% by supplementing the maize-based diet with 1 g P as MCP, but no further improvement was achieved with 2 g P as MCP. Chickens given the heated triticale-based diets, still containing some phytase (135 to 200 PU), displayed a FCR 25% lower than chicks on the unsupplemented maize-based diet with no further improvement with raw triticale-based diets.

Improvements in weight gain, feed intake, and FCR with increased available P provision through P or phytase supplementation of low P diets have been previously reported (Broz *et al.*, 1994; Kornegay *et al.*, 1996; Sebastian *et al.*, 1996a and b; Paik, 2003). From a literature review including 298 observations from 18 literature references, Lescoat *et al.* (2005) established an exponential relationship between body weight gain of chickens slaughtered at 21 to 24 d and P intake. The two-fold increase in final weight with the addition of 2 g P as MCP in the maize-based diet fits well with the relationship established by these authors. Increased weight gain with supplemental P may have resulted not only from an increase in feed intake but also from a specific effect of P on growth. A specific effect of P is expected in the chick because of the important role of this element in the formation of the skeletal system and in body metabolism (*e.g.* nucleic acids, high-energy compounds and various enzymatic reactions), and because of the fast rate of chick growth and its low capacity of P storage (Kornegay *et al.*, 1996).

Bone FF DM followed a trend very similar to final weight (r = 0.90, *P*<0.001, n = 90) with an increase in bone FF DM by 2.9 times when 2 g of P were added to the maize based diet. Bone FF DM responded linearly to P added (*P*<0.001) and linearly (*P*<0.001) and quadratically (*P*<0.01) to phytase activity. The amplitude of the response of bone ash (g) was a 5.1-fold increase when 2 g of P were added to the maize based diet. Bone ash (g and relative to FF DM) increased linearly with added P and with phytase activity (P<0.001) and quadratically with phytase activity (P<0.001). The linearity and the higher amplitude of the response of bone ash to dietary P compared to body weight gain indicates the ability of bone to incorporate P beyond the dietary supply needed to maximize weight gain (Figure). Relying on 316 observations collected in 15 literature references, Lescoat *et al.* (2005) established that bone ash concentration in chickens reached a plateau when 250-300 mg total P were ingested per d, a value that is higher than the 220 mg total P daily ingested by the chickens fed on the maize diet supplemented with 2 g P as MCP. Moreover, the increase in bone ash concentration fits well with the range of variation observed by these authors for similar dietary P supply. The improvement in bone ash in chickens fed on triticale diets indicates that plant phytase was effective in releasing P from the phytate-mineral complex. The maximum responses appeared to occur at around 600 PU plant phytase/kg diet whatever the indicator.

Figure near here

Before equivalency values of plant phytase for P as MCP can be estimated, the question arises whether responses of performance and bone characteristics observed in the current study can be ascribed solely to the improvement in available P supply by means of P as MCP in maize-based diets and by means of increased phytase activity in triticale-based diets. In the control diet, maize was preferred to a mixture of heated triticale batches to avoid any side effect due to heating other than lowering phytase activity and because maize is the reference cereal used when evaluating any source of phosphorus in the literature. The results of the current study cannot be used to compare the relative efficiency of maize and triticale for chick performance. Nevertheless, maize- and triticale-based diets provided sufficients amounts of all nutrients to fulfill the requirement of growing chicks up to 3 weeks of age, except for P (INRA, 1989). Particularly, because all diets were balanced for Ca, there is a very high probability that the response of bone ash concentration was related to available P supply rather than to any other dietary parameter. The magnitude of variation in performance in the current study is very large compared with the slight decrease in performance reported when maize is replaced by wheat (Singh *et al.,* 2003) or by triticale (Proudfoot and Hulan, 1988) in isocaloric, isonitrogenous, iso-Ca and iso-P diets fed to chickens. The former authors ascribe this depressed performance to the presence of arabinoxylans in wheat. In the current study, RAV in triticale-based diets was not greater than 1.40 ml/g, and, according to Maisonnier *et al.* (2001), such concentrations of RAV do not influence performance in chicks.

Non-linear models of the response of feed intake, final weight and bone characteristics to dietary mineral P and plant phytase activity as well as the derived equivalency values of P as MCP for plant phytase are presented in Table 6. Models were adjusted with a coefficient of determination of 0.77 to 0.89. All the indicators used were sensitive to plant phytase or MCP supply, but the fit was better for bone ash than for the other parameters. Moreover, the actual weight of bone ash displayed the highest R^2 for both the non-linear and polynomial adjustments. Other authors have concluded that bone ash weight is the most sensitive indicator to assess phytase efficacy in chickens (Zhang *et al.*, 2000; Hall *et al.*, 2003). Random variability in the organic matrix left after water and lipid removal may be responsible for the decreased R^2 value for ash expressed on a fat-free dry matter basis (Hall *et al.*, 2003). Based on bone ash parameters, increase in plant phytase activity from 0 to 250 PU was estimated to be equivalent to 0.46-0.47 g P as MCP. Between 250 and 500 PU, 250 PU were estimated to allow the release of an amount of P equivalent to 0.20-0.22 g of P as MCP. Between 500 and 1000 PU, an equivalency of 0.12-0.15 g P as MCP for 500 PU was obtained.

Table 6 near here

The equivalency values are not easy to compare with literature data because the efficacy of phytase has been shown to depend on several parameters including the dietary non-phytic P and Ca concentrations. These equivalency values may be relatively high because the triticale-based diets were not supplemented with P. Kornegay *et al.* (1996) demonstrated that, probably because of an available P supply closer to the requirement, the equivalency values decrease when the dietary non-phytate P concentration increases. On the contrary, the wide Ca:P ratio in the current study, greater than 2.8:1, may have reduced the efficacy of phytase in releasing phytate P. Increasing the Ca:P ratio in poultry diets was reported to decrease the efficacy of microbial phytase (Schoner et al., 1993; Qian *et al.*, 1996; Sebastian *et al.*, 1996b), with the optimum responses to phytase obtained at a Ca:P ratio as low as 1.1:1.

In accordance with our results, Paik (2003) observed that 650 PU of plant phytase achieved by the addition of wheat and wheat bran in a maize-soybean meal-based diet were equivalent to at least 1 g P as tricalcium phosphate in terms of growth performance and mineral retention in chickens up to 35 d. Similarly, an equivalency of 0.65-1 g P as MCP for 500 PU was reported by Frapin (1996) when evaluating plant phytase efficiency from various wheat varieties. At variance with our results, Juanpere *et al.* (2004) did not observe any effect of around 150 units of phytase from barley on performance and toe ash concentration in chicks. This absence of efficacy of plant phytase may be ascribable to a higher non-phytate P concentration than in the current study. From literature data, Kornegay *et al.* (2001) estimated that 500 PU of microbial phytase (3-phytase) were equivalent to 0.8 g P as MCP for 500 PU in terms of mineral retention in broilers. This higher figure may originate from the lower efficacy of plant phytase compared to microbial 3-phytase demonstrated in broilers (Frapin, 1996; *Potkansky*, 2000) and in pigs (Zimmermann *et al.*, 2002). The activity of plant phytase decreases rapidly when the pH decreases below the optimum pH of 5.0-5.5, whereas the 3-phytase still displays an activity of 60% of the optimum at pH 2 (Eeckhout and De Paepe, 1996). Plant phytase would be 40 to 80% less efficient in releasing P from phytates *in vivo* than 3-phytase due to the low pH in the stomach of pigs and gizzard of poultry (Frapin, 1996; *Potkansky*, 2000; Zimmermann *et al.*, 2002). The acidic pH of the gizzard inactivates plant phytase in contrast to protected microbial phytase and consequently plant phytase activity is limited to the crop (Frapin, 1996). Moreover, plant phytase may be more sensitive to the presence of pepsin than 3-phytase (Rapp *et al.*, 2001).

The present study confirmed the usefulness of the introduction of triticale in chickens' diets to reduce P emission and to limit environmental pollution, 500 PU of plant phytase being equivalent to 0.66-0.69 g P as MCP. Accounting for the genotypic variability of phytase activity in triticale (447 to 1843 PU/kg), a diet containing 450 g/kg triticale may display a phytase activity of 201 to 829 PU/kg, provided plant phytase is not denaturated during feed processing. In such a diet, P supplementation as MCP may be reduced by 0.4 to 0.8 g/kg, compared to a low phytase diet, such as a maize-based diet.

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Table 1. *Physical and chemical characteristics*[1] *of 30 batches of triticale*

Variety[2]	Origin	TGW	SW	HFN	DM	N	P	Phytic P	PA	PAV	RAV
		g	kg/hl	s	g/kg	g/kg	g/kg	g/kg	PU/kg	mL/g	mL/g
Trimaran	France	45.0	70.7	76	865	15,9	2.63	1.53	1023	2.35	1.79
A	France	47.0	69.8	67	871	16,1	3.11		987	2.89	1.84
Capitale	France	46.5	74.8	62	869	15,7	2.83	2.12	1226	3.42	1.76
Vision	Germany	57.5	75.5	109	874	16,1	3.13		974	3.56	2.28
B	Germany	48.5	72.3	84	856	16,1	2.75		842	3.92	2.01
Galtjo	Netherlands	55.0	75.2	260	857	17,1	3.22		728	3.84	1.97
Ego	Netherlands	53.5	73.9	158	857	15,0	3.11	2.32	1287	4.57	1.94
C	Sweden	45.5	73.0	70	853	14,6	2.83		881	3.50	1.83
D	Slovakia	53.0	72.2	64	862	17,2	2.75		1098	3.22	1.88
Colina	Romania	58.5	71.0	62	858	16,1	2.83	2.62	1346	2.44	2.07
E	Romania	54.5	74.5	75	859	17,8	3.57		1067	4.65	2.86
F	Poland	47.5	73.9	224	859	18,1	3.29	2.54	691	4.61	2.36
G	Poland	56.0	73.2	68	853	16,6	2.91		993	3.74	2.20
Binova	Germany	41.5	70.8	146	850	14,9	2.98		722	4.24	2.28
H	Germany	54.0	72.5	92	858	14,9	3.21		970	2.57	2.01
I	Poland	48.0	71.4	65	858	16,6	2.36		807	3.20	2.19
J	Poland	54.0	73.4	68	867	16,9	3.13		731	3.12	2.78
K	Poland	46.0	73.6	97	869	17,8	2.96	2.58	447	3.29	2.07
L	Poland	54.5	73.4	67	868	14,4	2.74		848	4.43	2.40
M	Slovakia	50.0	69.3	114	870	17,7	2.73		880	3.20	2.35
Aubrac	France	40.5	73.2	92	874	15,4	2.50	1.50	1103	3.86	2.32
Calao	France	41.5	68.1	62	858	16,1	2.83	1.95	1700	3.97	2.70
DI34-2	France	54.0	71.5	103	861	16,5	2.93	2.21	768	2.95	2.45
N	France	43.0	66.7	66	865	17,5	2.53	2.07	1843	3.52	2.06
O	France	49.0	74.4	94	859	15,4	2.06		1604	3.12	1.71
P	France	55.5	73.0	64	855	16,4	2.89		1064	2.87	2.01
Q	France	49.5	74.6	119	871	14,6	2.54		803	4.47	2.63
R	France	52.0	75.3	130	873	16,2	2.65	2.54	638	2.72	2.01
S	France	53.0	71.3	65	864	14,1	2.62		1101	4.12	1.98
T	France	52.0	71.3	68	873	16,4	3.21		1360	3.51	1.90
Mean		50.2	72.4	96	863	16,1	2.86	2.18	1018	3.53	2.15
SD		5.0	2.1	48	7	1,09	0.31	0.40	319	0.66	0.31
Min		40.5	66.7	62	850	14,1	2.06	1.50	447	2.35	1.71
Max		58.5	75.5	260	874	18,1	3.57	2.62	1843	4.65	2.86

[1]TGW, thousand grain weight; SW, specific weight; HFN, Hagberg falling number; DM, dry matter; PA, phytase activity; PAV, potential applied viscosity; RAV, real applied viscosity.

[2] cultivars or selection lines.

Table 2. *Composition and analytical characteristics of the experimental diets*

Diet	1 to 3	4 to 12 [1]		1 to 3	4 to 12 [1]
Ingredients	(g/kg diet as fed)		Analytical characteristics	(/ kg diet)	
Maize	450	0	Metabolisable energy, MJ [4]	13.2	13.3
Soybean meal	230	230	Protein (N x 6.25), g [4]	216	216 to 219
Soy isolate	70	60	Crude fibre, g [4]	42 to 40	36
Triticale	0	450	Lysine [4]	12.0	12.0
Maize starch	110	125	Methionine + Cystine [4]	9.0	9.0
Wheat straw [2]	59 to 54	39	Ca, g [4]	10.6	10.6
Vegetable oil	45	60	P, g [5]	3.5 to 5.7	3.2 to 3.8
Calcium carbonate [2]	25 to 21	25	Phytic P, g [4]	2.2	2.0 to 2.3
Monocalcium phosphate [2]	0.0 to 8.8	0.0	Phytase activity, PU [5]	<50	135 to 1390
DL-methionine	2.0	2.0	RAV, mL/g [5]	0.63 to 0.80	0.96 to 1.40
Sodium chloride	3.0	3.0			
Minerals and vitamins premix [3]	6.0	6.0			

[1] Without heat treatment, variety DI34-2, Trimaran, Aubrac and Calao in diets 4, 5, 6 and 7, respectively; heat-treated, variety DI34-2, Trimaran, Aubrac and Calao in diets 8, 9, 10 and 11, respectively; variety Calao, 200 g/kg without heat treatment and 250 g/kg heat-treated in diet 12. Heat treatment, which aimed at reducing phytase activity, consisted in two successive heating in a microwave oven at 600 W for 2 minutes.

[2] Wheat straw: 59, 56 and 54; calcium carbonate, 25, 23 and 21; monocalcium phosphate, 0.0, 4.4 and 8.8 g/kg in diets 1, 2 and 3, respectively.

[3] Vitamin-trace mineral mix that provided the following per kg diet: vitamin A (retinyl acetate), 10000 IU; vitamin D3 (cholecalciferol), 4000 IU; vitamin E (DL-alpha-tocopherol), 80 mg; vitamin K3 (menadione), 4 mg; vitamin B1 (thiamin), 4 mg; vitamin B2 (riboflavin), 6.4 mg; vitamin B3 (PP, niacin), 80 mg; vitamin B5 (Ca pantothenate), 20 mg; vitamin B6 (pyridoxine), 5.6 mg; vitamin B8 (biotin, H), 0.2 mg; vitamin B9 (folic acid), 2.4 mg; vitamin B12 (cyanocobalamin), 0.016 mg; choline, 440 mg, Fe ($FeSO_4$), 40 mg ; Cu ($CuSO_4$), 16 mg; Mn (MnO), 64 mg; Zn ($ZnSO_4$), 72 mg; Co ($CoSO_4$), 0.5 mg; I ($Ca(IO_3)_2$), 1.6 mg; Se (Na_2SeO_3), 0.16 mg.

[4] Estimated from INRA (1989) and the analysed N and phytic P concentrations in the four varieties of triticale.

[5] Analysed according to the methods described in the materials and methods section: P, 3.5, 4.5, 5.7, 3.8, 3.2, 3.6, 3.6, 3.7, 3.3, 3.3, 3.7 and 3.8 g/kg; phytase activity, < 50, < 50, < 50, 620, 875, 920, 1390, 180, 200, 180, 135 and 645 PU/kg; RAV, Real Applied Viscosity, 0.76, 0.63, 0.80, 1.04, 0.96, 1.08, 1.03, 1.16, 1.13, 1.40, 1.22 and 1.02 ml/g in diets 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12, respectively.

Table 3. *Correlation coefficient* (r)[1] *between physical and chemical characteristics*[2] *of the 30 batches of triticale*

	TGW	SW	HFN	DM	N	P	Phytic P[3]	PA	PAV
SW	0.42 (*)								
HFN	0.04	0.38 (*)							
DM	-0.05	0.09	-0.14						
N	0.05	-0.11	0.19	0.05					
P	0.35	0.20	0.31	-0.11	0.32				
Phytic P[3]	0.66 (*)	0.34	0.40	-0.27	0.45	0.60(*)			
PA	-0.15	-0.46 (*)	-0.38 (*)	-0.05	-0.10	-0.27	-0.36		
PAV	0.06	0.00	0.03	0.05	0.21	0.29	-0.03	-0.18	
RAV	-0.13	0.21	0.35	-0.13	-0.12	0.23	0.03	-0.03	0.43 (*)

[1] *, *P*<0.05.

[2]TGW, thousand grain weight; SW, specific weight; HFN, Hagberg falling number; DM, dry matter; PA, phytase activity; PAV, potential applied viscosity; RAV, real applied viscosity.

[3] n = 11.

Table 4. *Phytase activity in 5 varieties of triticale*

Variety	n	Phytase activity, PU/kg [1]
DI34-2	4	1012 ± 102 a
Aubrac	6	1320 ± 87 b
Trimaran	6	1424 ± 125 b
Capitale	4	1815 ± 126 c
Calao	6	2146 ± 145 d
P		<0.001
RSD[2]		119

[1] values are means ± standard deviation.

[2] RSD, residual standard deviation.

a-d; Means within a column not followed by the same letter differ at $P<0.05$.

Table 5. *Growth performance and bone[1] characteristics in chickens given the maize-based diets supplemented with graded levels of P as monocalcium phosphate or the triticale-based diets containing graded levels of phytase[2]*

Diet		Plant phytase PU/kg diet	n	Initial weight g	Final weight[3] g	Feed intake[3] g	FCR[3, 4]	Bone DM[3, 4] g	Bone ash[3, 4] g	Bone ash g/kg FF DM[3, 4]
					Maize-based diets					
	P as monocalcium phosphate (g/kg)									
1	0	< 50	5	154	411 a	594 a	2.33 a	1.00 a	0.214 a	215 a
2	1	< 50	7	151	747 d	982 c	1.65 cd	2.02 c	0.589 c	292 f
3	2	< 50	8	150	901 e	1225 d	1.63 d	2.88 d	1.090 d	379 g
					Triticale-based diets					
	45 raw triticale									
4	DI-34-2	620	7	154	764 d	1039 c	1.70 bc	2.07 c	0.587 c	285 ef
5	Trimaran	875	8	151	751 d	1055 c	1.76 bc	1.98 c	0.524 c	266 de
6	Aubrac	920	8	150	755 d	1032 c	1.71 bc	2.00 c	0.572 c	285 ef
7	Calao	1390	8	151	737 d	1045 c	1.79 bc	1.99 c	0.542 c	272 ef
	450 g/kg heated triticale									
8	DI-34-2	180	7	151	620 c	739 b	1.77 bc	1.46 b	0.364 b	251 cd
9	Trimaran	200	8	152	536 b	646 ab	1.84 b	1.27 ab	0.301 b	237 bc
10	Aubrac	180	8	150	539 b	693 ab	1.79 bc	1.37 b	0.327 b	238 bc
11	Calao	135	8	150	567 bc	706 ab	1.68 c	1.43 b	0.330 b	231 ab
	Raw (200 g/kg) and heated (250 g/kg) triticale									
12	Calao	645	8	153	712 d	959 c	1.72 bc	1.92 c	0.504 c	264 d
P					<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
RSD[4]					65	102	0.15	0.25	0.071	19

[1] Right tibiotarsus.

[2] Values are given on the basis of one cage of two birds.

[3] Linear (L) and quadratic (Q) effects of P added as monocalcium phosphate (g/kg diet) and of phytase activity in the diet (PU/kg diet):

Final weight: P added, L ($P<0.001$), Q ($P<0.01$); phytase activity, L ($P<0.001$), Q ($P<0.001$), RSD = 69, $R^2 = 0.77$.

Feed intake: P added, L ($P<0.001$), Q ($P<0.05$); phytase activity, L ($P<0.001$), Q ($P<0.001$), RSD = 104, $R^2 = 0.78$.

FCR: P added, L ($P<0.01$), Q ($P<0.05$); phytase activity, L ($P<0.001$), Q ($P<0.01$), RSD = 0.19, $R^2 = 0.23$.

Bone DM: P added, L ($P<0.001$); phytase activity, L ($P<0.001$), Q ($P<0.001$), RSD = 0.25, $R^2 = 0.78$.

Bone ash (g): P added, L ($P<0.001$); phytase activity, L ($P<0.001$), Q ($P<0.001$), RSD = 0.075, $R^2 = 0.90$.

Bone ash (g/kg FF DM): P added, L ($P<0.001$); phytase activity, L ($P<0.001$), Q ($P<0.001$), RSD = 20, $R^2 = 0.81$.

[4] DM, dry matter; FF DM, fat-free dry matter; FCR, feed conversion ratio; RSD, residual standard deviation.

a-g; Means within a column not followed by a common letter differ at $P<0.05$.

Table 6. *Adjustment of final weight, feed intake, bone[1] dry matter and bone ash to P as monocalcium phosphate (MCP, g/kg diet) and phytase activity (PU/kg diet) and equivalency values of P as MCP (g) for plant phytase (PU)*

	Final weight[3] g	Feed intake[3] g	Bone FF DM[4, 6] g	Bone ash[4] g	Bone ash[4] g/kg FF DM[6]
Non-linear adjustment[2]					
Coefficients [2]					
a	409	553	0.996	0.180	211
b	621	990	0.958	0.446	83.3
c	353	571	1.09	0.393	67.1
l	0.786	0.568			
k	0.00352	0.00200	0.00281	0.00303	0.00338
R^2	0.77	0.77	0.77	0.89	0.81
RSD	67	105	0.25	0.075	20
Equivalency values [5]					
Plant phytase (PU)	P as monocalcium phosphate (g)				
150	0.34	0.29	0.42	0.32	0.32
250	0.52	0.45	0.60	0.47	0.46
500	0.81	0.80	0.89	0.69	0.66
750	0.96	1.05	1.02	0.79	0.74
1000	1.02	1.22	1.08	0.84	0.78
1250	1.05	1.33	1.11	0.86	0.79

[1] Right tibiotarsus.

[2] Models were generated using treatment means, R^2 (coefficient of determination) and RSD (residual standard deviation) are calculated relative to individual observations.

[3] The model was $Y = a + b\,(1 - e^{-l\,MinP}) + c\,(1 - e^{-k\,Phyt})$, with Y = response measurement, MinP = P added as MCP (g/kg diet), Phyt = phytase activity (PU/kg diet).

[4] The model was $Y = a + b\,MinP + c\,(1 - e^{-k\,Phyt})$, with Y = response measurement, MinP = P added as MCP (g/ kg diet), Phyt = phytase activity (PU/kg diet).

[5] Final weight, feed intake, calculated as $MinP = -1/l\ \ln\,[1 - A\,(1 - e^{-k\,Phyt})]$; Bone dry matter and bone ash, calculated as $MinP = A\,(1 - e^{-k\,Phyt})$, with A = c/b, MinP = P as MCP (g), Phyt = phytase activity (PU)

[6] FF DM, fat-free dry matter.

Figure heading

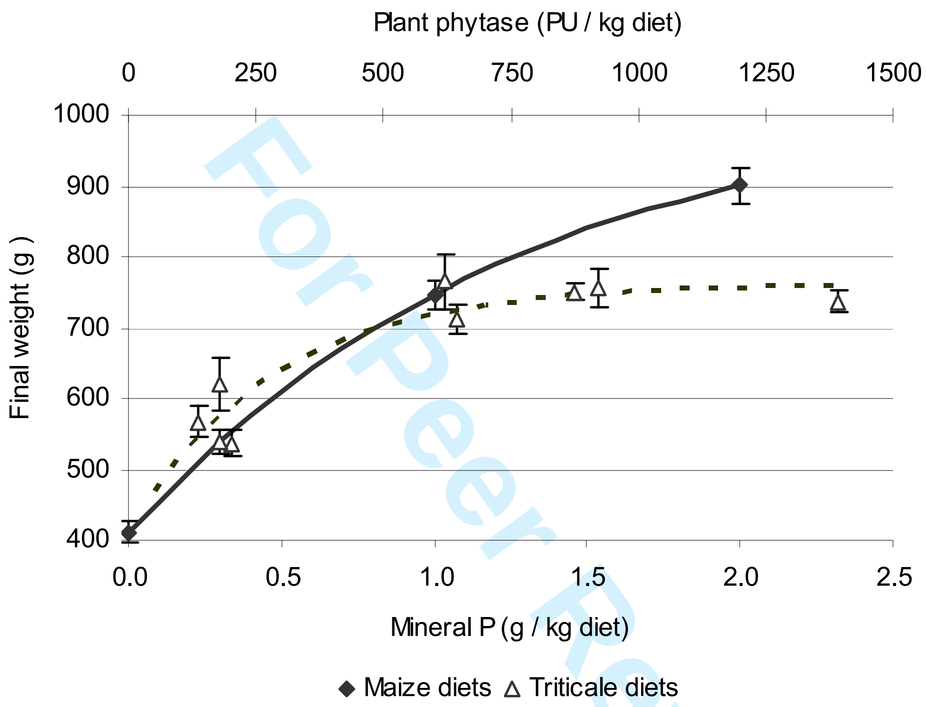
Figure. *Response of final weight and bone[1] ash to P as monocalcium phosphate in maize based diets and to plant phytase in triticale based diets.*

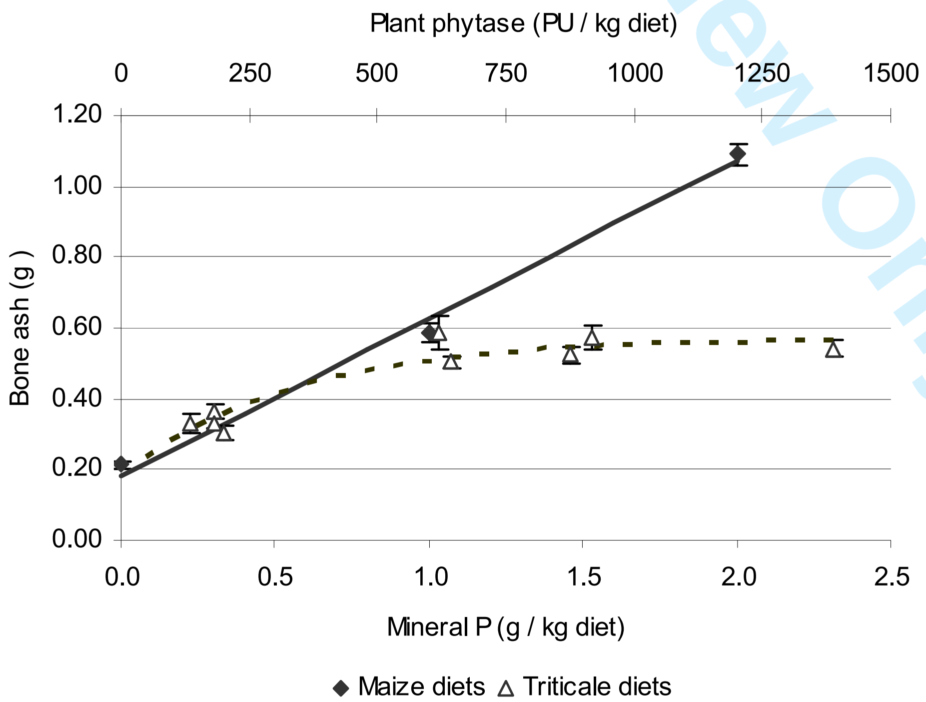
Figure footnotes

[1] Right tibiotarsus.

Values are means ± S.E.

Parameters of the models are presented in Table 6.


Plant phytase (PU / kg diet)
0 250 500 750 1000 1250 1500
1000 900 800 700 600 500 400
Final weight (g)
0.0 0.5 1.0 1.5 2.0 2.5
Mineral P (g / kg diet)
Maize diets Triticale diets


Plant phytase (PU / kg diet)
0 250 500 750 1000 1250 1500
1.20 1.00 0.80 0.60 0.40 0.20 0.00
Bone ash (g)
0.0 0.5 1.0 1.5 2.0 2.5
Mineral P (g / kg diet)
Maize diets Triticale diets