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Samenvatting NL
Dit experiment is uitgevoerd met als doel de behoefte aan opneembaar fosfor (oP) bij huidige vleeskuikens vast te stellen op basis van een dosis-respons studie. Deze studie geeft aan dat het voer voor het realiseren van maximale dierprestaties in fase 1 (d1-d10) 5,4 g oP per kg dient te bevatten, in fase 2 (d11-d21) 4,3 g oP per kg en in fase 4 (d31-d38) 2,6 g oP per kg. In fase 3 (d22-d30) had het oP-gehalte geen effect op de dierprestaties. Voor het realiseren van een maximaal tibia-asgehalte was in deze fase een oP-gehalte van 3.8 g/kg noodzakelijk.

Summary UK
The aim of the current experiment was to determine the aP requirements in modern broilers by performing a dose-response experiment. This study showed that for realizing maximal performance, in phase 1 (d1-d10) 5.4 g aP per kg of feed was required, in phase 2 (d11-d21) 4.3 g aP per kg of feed, and in phase 4 (d31-d38) 2.6 g aP per kg of feed. In phase 3 (d22-d30), performance of the broilers was not affected by dietary aP content. For realizing maximal tibia ash content in phase 3, an aP-level of 3.8 g/kg was required.

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Preface

Feed4Foodure is a public-private partnership between the Dutch Ministry of Economic Affairs, a consortium of various organizations within the animal production chain, and Wageningen UR Livestock Research. Feed4Foodure aims to contribute to sustainable and healthy livestock farming in the Netherlands, simultaneously strengthening our competitive position on the global market.
On request of the Dutch Ministry of Economic Affairs, as well as of the Dutch Product Board Animal Feed, the current study was performed to investigate the absorbable phosphorus (aP) requirement in modern broilers.
For the current study, scientists of Wageningen UR Livestock Research worked together with representatives from the various private partners, including Agrifirm, ForFarmers, Nutreco, and De Heus. The authors thank the industry partners of the project team for their worthwhile input.

Dr. Gert van Duinkerken, leader Feed4Foodure program line “More with less; improving resource efficiency in the chain”.

Summary

Phosphorus (P) is one of the essential minerals for all kind of animals. It plays a key role in cellular metabolism, as a part of the energy metabolism of the cell (adenosine triphosphate production), in cellular regulatory mechanisms, and in bone development. An adequate P supply allows animals to attain their optimum genetic potential in growth, feed efficiency and skeletal development.

The current absorbable P (aP) recommendations of broilers in the Netherlands (CVB, 2012) are based on a report of Van der Klis and Blok (1997). Since then, however, performance level of broilers has been changed drastically. Broilers are continuously selected for high breast meat production and increased feed efficiency, which affects aP requirements. Van Krimpen et al. (2013) suggested that the aP recommendations of modern broilers is currently underestimated, especially at young age. Therefore, the aim of the current experiment was to determine the responses of broilers on incremental dietary P contents, and the consequences of these responses for the aP- requirements in modern broilers.

A trial was conducted to determine the aP requirements of male broiler chicks in four different periods: 1 to 10 (phase 1), 11 to 21 (phase 2), 22 to 30 (phase 3) and 31 to 38 (phase 4) days of age. The calcium/absorbable phosphorus ratio of all diets was kept constant at a ratio of 2.2:1. In total 6,510 Ross308 broilers were randomly allotted in a completely randomized design with 6 treatments, 5 replicate pens per treatment, and 217 birds per pen. In each phase, diets were provided with aP contents of 75%, 87.5%, 100%, 112.5%, 125%, or 137.5% of CVB (2012) recommendations. The calculated aP levels were based on CVB (2011) contents in feed ingredients. The experimental diets were mainly based on corn, wheat and soybean meal. The differences in Ca and aP content were realized by exchanging monocalciumphosphate and limestone by diamol, an inert filler ingredient without any nutritional value. Titanium dioxide was used as indigestible marker. The aP requirements were based on the aP intake levels necessary for realizing maximal body weight gain, feed conversion ratio and tibia ash contents, as estimated by a broken stick model.

The conclusions were as follows:

- In phase 1, 2 and 4, growth performance responded linear to incremental dietary aP contents.
- In phase 1 and 2, FCR responded linear to incremental dietary aP contents, whereas FCR in phase 4 responded quadratic.
- In phase 4, the absolute tibia breaking strength responded linear to incremental dietary aP contents.
- In phase 1 and phase 2, the calculated dietary aP-levels for maximal BWG and minimal FCR according to the broken stick model were higher than the calculated aP-level for maximal tibia ash content.
- In phase 3, performance of the broilers was not affected by dietary aP content. For maximal tibia ash content, an aP-level of 3.8 g/kg was required.
- In phase 4, the calculated dietary aP-levels for maximal broiler performance and tibia ash content were similar (2.6 g/kg).
- Broilers were able to increase dietary P absorption at aP-levels below 112.5% (d21 and d30) or 125% (d10 and d38) of the CVB (2012) recommendations. P absorption was increased by increasing phytate degradation.
- Tibia breaking strength, expressed per g tibia or per mm tibia section, was in most cases not affected by dietary aP-content. In 38 day-old broilers, the absolute tibia breaking strength, as well as the breaking strength expressed per mm tibia section, was lower in birds fed the 75% aP-diets compared to birds fed the other diets.
- Footpad lesions, feather quality, and litter quality were not affected by dietary aP-levels.
- Average carcass P content was 4.5, 4.8, 4.5, and 4.2 g/kg at d10, d21, d30, and d38, respectively. At d10 and d21, carcass P content tended to increase with increasing dietary aP-content.

- In birds dissected at d10 and d21, carcass Ca content increased with increasing dietary aP-content. At d30 and d38, carcass Ca content was not affected by dietary treatments; average carcass Ca content was 6.0 and 5.6 g/kg, respectively.

This study showed that for realizing maximal performance, in phase 1 (d1-d10) 5.4 g calculated aP per kg of feed was required, in phase 2 (d11-d21) 4.3 g calculated aP per kg of feed, and in phase 4 (d31-d38) 2.6 g calculated aP per kg of feed. In phase 3 (d22-d30), performance of the broilers was not affected by the calculated aP content. For realizing maximal tibia ash content in phase 3, an calculated aP-level of 3.8 g/kg was required. In phase 1 and 2 of the current study, the dietary aP level required for maximal performance phase are about 30% higher compared to the current CVB recommendations. For these phases, aP recommendations should be reconsidered.

Samenvatting

Fosfor (P) is voor alle diersoorten één van de essentiële mineralen. Het speelt een belangrijke rol bij de energiestofwisseling in het lichaam (productie van adenosine trifosfaat), cellulaire regulerende mechanismen en bij de botvorming. Een adequate P voorziening stelt dieren in staat om hun genetische potentie, voerefficiency en ontwikkeling van het skelet optimaal tot expressie te brengen.

De huidige Nederlandse aanbevelingen (CVB, 2012) voor opneembaar fosfor (oP) voor vleeskuikens zijn gebaseerd op een studie van Van der Klis en Blok (1997). Sinds die tijd is het prestatieniveau van vleeskuikens sterk veranderd. Vleeskuikens worden continu geselecteerd op een hogere productie van filet en op een verbetering van de voerefficiëntie, wat van invloed is op de oP-behoefte. Van Krimpen et al. (2013) veronderstelden dat de oP-behoefte van moderne vleeskuikens op dit moment wordt onderschat, met name aan het begin van de groeiperiode. Daarom is het huidige experiment uitgevoerd dat als doel had om de responsen van vleeskuikens op toenemende fosforgehalten in het voer vast te stellen en de gevolgen hiervan op de behoefte aan opneembaar fosfor bij het huidige vleeskuiken.

In dit experiment is de opneembaar fosforbehoefte (oP) van vleeskuikenhaantjes tijdens de vier fases van de groeiperiode vastgesteld: 1 tot 10 (fase 1), 11 tot 21 (fase 2), 22 tot 30 (fase 3) en 31 tot 38 (fase 4) dagen leeftijd. De calcium/opneembaar fosfor verhouding was voor alle fasen gelijk (2,2:1). In totaal werden 6.510 Ross 308 vleeskuikens willekeurig toegewezen aan 6 behandelingen, 5 herhalingen (hokken) per behandeling en 217 kuikens per hok. Per fase werden voeders verstrekt met respectievelijk 75%, 87,5%, 100%, 112,5%, 125% en 137,5% van de behoefte volgens CVB (2012). De oP niveaus van de voeders waren gebaseerd op de gehalten voor grondstoffen, zoals vermeld in de CVB Tabel (2011). De voeders waren hoofdzakelijk gebaseerd op maïs, tarwe en sojaschroot. De verschillen in calcium- en opneembaar fosforgehalte tussen de voeders werden gerealiseerd door het uitwisselen van monocalciumfosfaat en krijt met diamol. Deze laatste grondstof is een inert vulmiddel zonder enige voedingswaarde. Als onoplosbare marker werd titaandioxide (TiO_2) toegevoegd aan de voeders. De oP-behoefte werd afgeleid op basis van de oP-opname die nodig was voor het realiseren van maximale groei, voederconversie en tibia-asgehalte, zoals geschat door een gebroken lijnmodel.

De conclusies zijn als volgt:

- In fase 1, 2 en 4 nam de groei van de kuikens lineair toe met de stijging van het oP-gehalte van het voer.
- In fase 1 en 2 nam de voederconversie lineair af met de stijging van het oP-gehalte van het voer, terwijl er in fase 4 een kwadratisch verband was tussen het oP-gehalte en de voederconversie.
- In fase 4, nam de absolute breeksterkte van tibia lineair toe met de stijging van het oP-gehalte.
- In fase 1 en 2 waren de berekende oP-gehalten voor maximale groei en minimale voederconversie volgens het gebroken lijnmodel hoger dan de berekende oP-behoefte voor maximaal tibia-asgehalte.
- In fase 3 had het oP-gehalte geen effect op de dierprestaties. Voor een maximaal tibia-asgehalte was in deze fase een oP-gehalte van 3.8 g/kg noodzakelijk.
- In fase 4 was de berekende oP-behoefte voor maximale dierprestaties gelijk aan die voor maximaal tibia-asgehalte; deze bedroeg 2.6 g/kg.
- Om de fosforvoorziening naar het metabolisme in stand te houden, bleken vleeskuikens tot op zekere hoogte in staat om de P-opneembaarheid van het voer te verhogen. Dit mechanisme deed zich voor bij oP-niveaus onder 112.5% (dag 21 en dag 30) of onder 125% (dag 10 en dag 38) ten opzichte van de CVB-aanbevelingen (2012). De P-opneembaarheid steeg als gevolg van een toename in fytaatafbraak.
- De breeksterkte van de tibia, uitgedrukt per gram tibia of per mm tibia doorsnede, was in de meeste gevallen niet beïnvloed door het oP-gehalte van het voer. Alleen op dag 38 daalde de tibia breeksterkte bij kuikens die voer kregen met een oP-gehalte van 75% van de CVB aanbevelingen.

- Voetzoollaesies, kwaliteit verenkleed en strooiselconditie werden niet beïnvloed door het oP-gehalte van het voer.
- Het fosforgehalte in het karkas bedroeg gemiddeld 4,5, 4,8, 4,5 en 4,2 g/kg in kuikens die geslacht werden op respectievelijk dag 10, 21, 30 en 38. Op dag 10 en dag 21 was er een tendens tot hogere P-gehalten in het karkas bij oplopende oP-gehalten van het voer.
- In kuikens die geslacht waren op dag 10 en dag 21 nam het calciumgehalte van het karkas toe met oplopende oP-gehalten van het voer. Op dag 30 en dag 38 waren er geen effecten van het oP-gehalte op het calciumgehalte in het karkas. De calciumgehalten bedroegen toen respectievelijk 6,0 en 5,6 g/kg.

Deze studie geeft aan dat het voer voor het realiseren van maximale dierprestaties in fase 1 (d1-d10) 5,4 g berekend oP per kg dient te bevatten, in fase 2 (d11-d21) 4,3 g berekend oP per kg en in fase 4 (d31-d38) 2,6 g berekend oP per kg. In fase 3 (d22-d30) had het berekende oP-gehalte geen effect op de dierprestaties. Voor het realiseren van een maximaal tibia-asgehalte was in deze fase een berekend oP-gehalte van 3.8 g/kg noodzakelijk. De vastgestelde oP behoeften in fase 1 en 2 van deze studie zijn ongeveer 30% hoger dan de huidige oP normen volgens CVB (2012). Deze studie geeft aanleiding om de oP normen volgens CVB voor jonge vleeskuikens te heroverwegen.

1 Introduction

Phosphorus (P) is an essential mineral for all kind of animals. It plays a key role in cellular metabolism, as a part of the energy metabolism of the cell (ATP; adenosine triphosphate production), in cellular regulatory mechanisms, and in bone. Its influence on the animal metabolism is critical, because adequate P supply enables the animals to attain their optimum genetic potential in growth, feed efficiency and skeletal development. The three main issues concerning phosphorus management in poultry are i) the limited P store in the world, ii) the costs and iii) the excretion into the environment (Singh, 2008; Li et al., 2015).
For a sustainable P use, it is important to adjust the P supply to the P requirement of the animal. Therefore, knowledge of the P requirement of the animal is essential for manufacturing diets. However, overfeeding of dietary P is common practice in global poultry production, with excesses of 20 to 100% over published requirements (Applegate and Angel, 2014). The NRC Nutrient Requirements of Poultry (1994) has been a benchmark publication for the research, judicial, and regulatory communities, both in the US and abroad since the first published edition in 1944. However, Applegate and Angel (2014) argued that with extraordinary changes in growth and productive potential of modern poultry strains, as well as changes to body composition and egg output, it follows that nutrient needs have changed beyond what the bird can compensate for with increasing intake per unit of BW. Research publications used for phosphorus recommendations in the last NRC are now, at best, from 1991 and at worst from 1947 (Applegate and Angel, 2014).
In the Netherlands, the current absorbable P (aP) recommendations for broilers are based on a report of Van der Klis and Blok (1997). This aP system is based on the determination of retainable P, as well as on the amount of phytate degradation of feed ingredients. Absorbability of non-phytate P is assumed to be 80%. In this aP system, correction factors are used for feed ingredients that showed to have high levels of phytate degradation (>22%).
Since the report of Van der Klis and Blok (1997), however, performance level of broilers has changed dramatically (Havenstein et al., 2003; Collins et al., 2014), because broilers are continuously selected for high breast meat production, which changed the ratio between bones and soft tissues, and consequently aP requirements.
In terms of P storage, boilers largely differ from mammals, because only 55% of the body's total P in 17 d old broilers is stored in bone, versus 85% in mammals (Van Krimpen et al., 2013). These authors also concluded that the aP requirement of modern broilers is higher than the recommendations as suggested by Van der Klis and Blok (1997), especially in phase 1.
The aim of the current experiment was to determine the responses of fast growing (Male Ross 308) broilers on incremental dietary P contents, and the consequences of these responses for the aP-requirements in modern broilers.

2 Materials and Methods

2.1 Experimental animals

In this experiment 6,510 male broilers (Ross 308) were used and assigned to 30 pens (217 animals per pen). Only male broilers were used, because aP requirement is more critical in this gender compared to females. Day old chickens were supplied by the hatchery Probroed, the Netherlands. Birds were vaccinated against IB at the hatchery, against Newcastle Disease at 10 d of age, and Gumborro around 18 days of age. The broilers were slaughtered at d 39 by De Kuikenaer according to common practice.

2.2 Experimental design

The experiment was set up in a completely randomized block design with six different treatments and 5 replicate pens per treatment. Blocks consisted of 6 adjacent pens. The treatments ranged from 75 to 137.5% of the current recommendations (CVB, 2012). Based on a previous study of our group (Van Krimpen et al., 2013), it was expected that the aP requirement of these birds would be higher than current recommendations. Therefore, 2 levels below and 3 levels above the 100% level were included. Because exponential curve fitting was to be used, some levels below the expected requirement were necessary for appropriate modelling of the data.
Feed was composed in accordance to CVB (2012) recommendations to assure that all broilers' requirements for nutrients, apart from Ca and P, were met. In order to modify only the P and Ca content in the diet, monocalciumphosphate and limestone were exchanged by diamol, an inert filler ingredient without any nutritional value. In all diets, the Ca to absorbable P ratio was maintained constant at a ratio of 2.2:1, which is considered as the optimum ratio in broiler diets (Van Krimpen et al., 2013). All diets were produced as expanded and pelleted diets. The dietary treatments are presented below (Table 1).

Table 1
Absorbable phosphorus levels (g/kg) of dietary treatments per phase of age.

Treatment	Absorbable P level (% of CVB)	Phase 1 d0-10	Phase 2 d11-21	Phase 3 d22-30	Phase 4 d31-39
1	75.0	3.0	2.6	2.3	2.1
2	87.5	3.5	3.0	2.6	2.5
3	100.0[1]	4.0	3.4	3.0	2.8
4	112.5	4.5	3.8	3.4	3.2
5	125.0	5.0	4.3	3.8	3.5
6	137.5	5.5	4.7	4.1	3.9

1) The 100% level was based on the current CVB recommendations (CVB, 2012). CVB provides one aP recommendation (3.1 g/kg) for the period 11-30 d. Because in this experiment a 4-phase feeding system was used, the recommended aP content of 3.1 g/kg for d10 to d30 was subdivided in 3.4 g/kg for d11-21 and 3.0 g/kg for d22-30, which results in a similar aP consumption as compared to the 3-phase feeding system.

2.3 Experimental facilities

The experiment was carried out in one room of House 9 of Laverdonk, the experimental facility of Agrifirm in Heeswijk Dinther, the Netherlands, which was automatically controlled by a Fancom climate computer. Heating was provided by floor heating and by one additional heater. Three run-through fans ensured a good partitioning of the heat through the whole house. Ventilation was regulated by length ventilation. At arrival, birds received a light schedule of 20 h light and 4 h darkness. Light intensity was gradually reduced to 5/10 lux at d 11-14, and another darkness period of max 4 h was built in. The room temperature was set at approx. 33-35°C at the day of arrival, and thereafter gradually decreased with 1 degree Celsius to 20-18°C during the last production days.

Each pen had 13.5 m^2 effective floor surface (4.45 m x 3.14 m), and pens were separated from each other by plastic coated wire. At the day of arrival, ±12.5 kg of wood shavings was provided per pen. One empty pen per treatment (6 pens in total) was reserved for the birds used for the dissections. Twenty five birds (5 birds per pen x 5 pens with the same dietary treatment) were placed in this pen 5 days before the dissection. Birds were colour marked, so that it was known from which original pen they were collected. This pen had the same design as other pens, except for the wood shavings. No litter was used to prevent a possible influence on the ileal digestibility coefficients.
Birds were fed semi ad libitum, meaning that each day feeders should become almost empty. Feed was distributed over 5 feeders from one distribution point per pen. Water was provided ad libitum and was distributed via drinking nipples (30 nipples per pen).

2.4 Experimental diets

Different diets were provided in a four phase feeding program. Phase 1 was from d0 to d10, followed by a phase 2 from d11 to d21, a phase 3 from d22 to d30, and a phase 4 from d30 to slaughter at d39. Diet compositions were similar to commercial diets, except for aP contents. Contrasts in aP levels were realized by use of monocalciumphosphate and limestone, while Ca/aP ratio of all diets was fixed at 2.2. No microbial phytase was added to the diets. Intrinsic phytase activity of wheat was inactivated by a boa compacter (model 500 x 1500, 90 kW, Pelleting Technology Nederland B.V., Schijndel, the Netherlands), which is a device that processes feed with heat and moisture. After production, phytase activity was determined in phase 2 and phase 4 diets. In these diets, phytase activity was below the detection level, indicating that the intrinsic phytase activity was effectively inactivated.
Only the diets with the highest and lowest aP content were produced in the factory. The other diets were produced by mixing these two diets at the farm level, according to the mixing ratios mentioned in Table 2. The feeding system on the farm was equipped to produce these mixes very accurately. All

treatments switched to the next feeding phase at the same time, after the dissections on the last day of each phase had been conducted.

Table 2
Mixing ratios (%) of Diet 1 and to Diet 6 per treatment.

Treatment	Absorbable P level (% of CVB)	Portion diet 1	Portion diet 6
1	75.0	100	0
2	87.5	80	20
3	100.0	60	40
4	112.5	40	60
5	125.0	20	80
6	137.5	0	100

In all diets, 3 g/kg titanium (5 g/kg titanium dioxide; TiO_2) was added on top as a marker. Phase 1 feed was pelleted at a 2.2 mm screen and crumbled afterwards, whereas the other diets were pelleted at 3 mm diameter. Before providing to the birds, the batches of the products in this experiment were analysed by Nutricontrol BV (Veghel, The Netherlands). Dietary ingredients and calculated composition of the diets are given in Appendix 1.

2.5 Measurements

Starter weight
At the day of arrival, 500 birds were weighed to establish a mean starter weight of the birds, where after 217 birds were placed in each pen. An overview of the distribution of treatments over pens is given in Appendix 2.

Body weight (gain), feed intake, water intake
Every pen was equipped with an automatic weighing system linked with a computer. Individual BW was recorded, as well as total recordings per day. Average BW per day was calculated by the computer. At the end of the experiment all birds were weighted manually, because heavier birds avoid the use of the weighing system, and therefore the recorded weight is not representative for the whole flock. Feed and water intake were recorded per pen and per period (phase 1 to 4) and printed from the computer every day.

Mortality
Mortality was recorded daily. Every day, one person checked the pens and collected the dead animals, registered pen number, probable cause of death, and their weight. The possible causes of death included: unknown, the size of the animal (too small which affected the resistance of the bird, or too big which could cause sudden death such as a heart attack), leg failures, accidents, or yolk inflammation.

Foot pad lesions and gait score
Foot pad lesions were scored two times during the weeks 3 and 4 by two persons. These persons (always the same) randomly selected eight birds per pen and scored the foot injuries. The scale of the scoring ranged from 0 to 2, ranging from “no lesion” to “severe lesion”.
The gait score was performed during the last days before dissection, for all the birds placed in the pens without litter. This scoring was then performed on 25 birds per treatment, 150 in total. The scale of this scoring ranged from 0 to 5, and was based on the protocol of Kestin et al. (1992) and Welfare Quality (2009). Scoring a zero meant the birds could walk perfectly, even run. At the opposite, scoring a five meant the animals could not walk, even not stand up. Birds with score 1 had a slight defect, resulting in an uneven gait. Score 2 meant the birds could not walk perfectly, but they were still able to walk in an almost normal way; walking abnormality was difficult to define. A score 3 meant a

definite and identifiable walking abnormality. Score 4 meant the animal showed a severe walking abnormality and could only walk a few steps.

Litter quality and feather score

From the second week of the experiment onwards, litter quality and feather score were recorded twice a week (every Monday and Thursday). One person was giving the scores to have the same notation criteria and to avoid differences between observers. The litter quality was scored from 1 to 5, ranging from “very good” to “very bad”. The feathers quality (dirtiness) was scored at the same time. The scale ranged from 1 (“very clean”) to 5 (“very dirty”) as well.

Ileal digestibility measurements

At d 10, 21, 30 and 38, 5 birds per pen (25 birds per treatment, 150 birds in total) were killed by T61. Of these 5 birds, 3 were allowed to consume feed until the moment of dissection (90 birds in total), to ensure the availability of ileal digesta content. These birds were used to determine ileal Ca and P digestibility and for bone parameters. The two remaining birds per pen were fasted for three hours before being killed (60 in total) for determination of total body analysis.

The ileum, which was defined as the part of the small intestine from Meckel’s diverticulum until 2 cm before the split between ileum and caeca, was removed from the abdominal cavity. Digesta of 3 birds was sampled by flushing with water, where after it was stored on ice until all 3 birds per pen were sampled. For each dissection day, the digesta of 3 birds per pen were pooled to collect sufficient and representative amounts of digesta to conduct all chemical analyses. All feed and digesta samples were analysed for dry matter, titanium, Ca, and P. Additionally, feed and digesta samples of d21 and d38 were analysed for phytate.

Apparent ileal digestibility of nutrients (AID) was calculated using the following equation:

$$AID(\%) = (1 - [(Ti_{feed}/Ti_{ileum} \times N_{ileum}/N_{feed})]) \times 100$$

where Ti_{feed} and Ti_{ileum} represent titanium content in feed and ileal digesta respectively, and N_{feed} and N_{ileum} are the nutrients in feed and ileal digesta respectively. Once the availability was obtained per treatment, the analysed ileal absorbable phosphorus content could be calculated following the equation below:

analysed aP content (g/kg) = P digestibility x P content of the diet

Tibia measurements

From the same 3 birds per pen as used for ileal digestibility, the left tibia was used to measure tibia weight, diameter and bone strength. The strength of the bones was determined by an “Instron 5564” machine, which pressed the bones until they break. The maximal extension load for breaking a tibia was used to evaluate the bone strength.

The right tibia of those 3 birds was used to determine the ash content in a pooled sample.

From the remaining 2 birds in which the tibia were still present?, the empty body (gut emptied by slightly squeezing) was frozen at -20°C. At a later time, they were grounded and analysed for Ca and P content.

Analytical Procedures

After defrosting, digesta samples were homogenized. All samples were analysed in duplicate. For determination of the DM content in digesta, samples were freeze-dried according to International Organization for Standardization (ISO) method number 6496 (1998). Following freeze-drying, samples were ground to pass a 1 mm screen and kept for analysis. Air-dry samples were dried in a forced air oven at 103^0C to a constant weight according to ISO 6496 (1998). Kjeldahl nitrogen content in feed was measured according to ISO 5983 (1997) in fresh samples. CP content was calculated as nitrogen * 6.25. For determining crude ash content, feed samples were incinerated at 550°C in a muffle furnace according to ISO 5984 (2002). Titanium oxide was determined according to the method developed by Short et al. (1996) and further refined by Myers et al. (2004). This method is based on digestion of the sample in sulphuric acid and addition of hydrogen peroxide to produce an intense orange/yellow colour that is read colorimetrically at 408 nm by use of an UV- visible spectrophotometer (Varian, CARY 50 probe). Ca and P contents in feed and ileal digesta were

determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) (ISO 11885, 1998).
The analytical method for determination of phytate de-phosphorylation (‘phytase’) activity is usually based on the colour that is formed upon the reaction of molybdate with the amount of inorganic P released from phytate after incubation of the sample with microbial phytase in an citrate acid buffer solution of pH 2.5 (Marounek et al., 2011). Inorganic P is responsible for the decolouration of ammonium molybdate. Phytase activity was measured as described by Eeckhout and De Paepe (1994). Briefly, 0.2 g of a finely ground sample was weighed in a 50 ml volumetric flask, which was then filled with a solution of 1.5 mM Na-phytate in 0.25 M acetate buffer, pH 5.5. The flasks were shaken for 15 min and then placed in a water bath at 37°C. After 10 min, a portion of the incubate (2 ml) was placed in a test tube, and the same volume of 10% trichloroacetic acid was added to stop the reaction. After another 60 min, the procedure was repeated. The contents of both tubes were centrifuged (5000 g, 10 min), and the concentration of P in the supernatant was determined using the acid molybdate reagent. Colour was measured in a spectrophotometer at 820 nm and compared with a calibration series containing 1 ml of 10% trichloroacetic acid per cuvette, 1ml of phosphate standard solution (0, 10, 20, 30 or 40 μmol/ml) and 2 ml of colour reagent. The increased concentration of P during the 60 min incubation was attributed to phytate de-phosphorylation activity. A unit of this activity (U) is defined as the activity that liberates P at a rate of 1 μmol/min.

2.6 Statistical analyses

The experiment was set up as a complete randomised block design in which the 6 treatments were arranged in 5 randomized blocks, consisting of 6 adjacent pens. Prior to analysis, data were checked for outliers using scatter plots and the Dixon-test for outliers. Before outliers were excluded, the numbers were checked.

Scale weights registered by the automatic weighing system were used to measure body weight in phase 1 (d0-10), phase 2 (d11-21), and phase 3 (d22-30), where pen weight, measured during depopulation, was used for body weight in phase 4 (d31-39), and the whole experimental period.

In phase 1 to 4, performance data were corrected for mortality and weight at start of the period.

Performance data (growth, feed and water intake, water to feed ratio, feed conversion ratio) were analysed according to the following model using the mixed procedure of the statistical package SAS (SAS Institute Inc., Cary, NC, USA, version 9.2). Other parameters such as weight at section days, overall litter quality, overall feather score, foot pad lesion, mortality, bone parameters, and P and Ca content in ileal digesta, tibia, and total body were averaged per pen and analysed the same way as the performance results. Two models were used for the overall statistical analysis. Model 1 was used to test the differences between diets in case of absence of repeated measurements:

$Y_{ij} = \mu + trt_i + e_i$

Where:

μ	=	average
trt	=	treatment (n=6)
e	=	residual error

Block (n=5) was included in the model as random term.

If an overall statistical treatment effect was found (P value < 0.05), a Fisher protected t-test was used to analyse pairwise differences. Pairwise differences are marked with a letter in superscript. Differences among means with $0.05 < P < 0.10$ were accepted as representing tendencies to differences.
Dose response graphs were composed for all the measured parameters. In these graphs, the Y-axis represents the response of interest and the X-axis the calculated dietary aP content, or the aP content analysed in this experiment .

In case of linear or quadratic responses on aP supplementation, a broken-line (linear-plateau) model (Genstat; R2LINES procedure) was used to estimate the aP requirement. In this model, the X-axis value at the intersection of the two lines represents the aP requirement for the concerning response criterion. In general, the aP-requirement estimated by a linear-plateau model is lower than estimated by a quadratic response model. The estimated requirement by use of a linear-plateau model roughly represents the requirement of an average broiler in the flock, whereas the aP requirement based on a quadratic response model represents the requirement of the best performing birds in the flock. Oversupply of P results in a decrease in P-efficiency. In the current study, therefore, the use of a linear plateau model was preferred, thereby preventing spoilage unwanted P-excretion to the environment.

3 Results

3.1 General

The experiment is performed in accordance with the proposed protocol, and no abnormalities were detected. Overall performance data of broilers during the experimental period were satisfying (Table 3) and met the standards of Ross 308 male performance data (Ross 308, 2012).

Table 3
Performance data of broilers during the experimental period from 0 to 39 days of age.

Performance data	Trial	Ross 308 performance data
Body weight day 0 (g)	38	42
Body weight day 39 (g)	2,632	2,666
Body weight gain day 0-39 (g/d)	67.5	68.4
Feed intake day 0-39 (g/d)	111.1	112.4
Feed conversion ratio	1.647	1.644
Mortality (%)	3.02	n.a.[1]

1) not available

3.2 Calculated and analysed nutrients of the diets

In Table 4, the calculated and analysed nutrients of the diets (g/kg, as fed basis) are provided. Only the diets with the highest (137.5%) and lowest (75%) aP content were produced and analysed. The other diets were produced by mixing these two diets at farm level.

Table 4

Calculated and analysed nutrients of the experimental diets (g/kg, as fed basis).

		Phase 1 diets		Phase 2 diets		Phase 3 diets		Phase 4 diets	
Nutrient	Method	75% aP-level	137.5% aP-level	75% aP-level	137.5% aP-level	75% aP-level	137.5% aP-level	75% aP-level	137.5% aP-level
Dry matter	Calc.	886	887	883	884	883	884	883	884
	Anal.	*905*	*909*	*907*	*909*	*906*	*907*	*898*	*908*
Ash	Calc.	75	76	65	65	60	60	58	58
	Anal.	*70*	*71*	*63*	*64*	*59*	*59*	*56*	*56*
Crude protein	Calc.	220	220	195	195	194	194	191	191
	Anal.	*224*	*226*	*204*	*200*	*204*	*201*	*196*	*197*
Crude fat	Calc.	72	72	71	71	78	78	82	82
	Anal.	*71*	*73*	*71*	*71*	*77*	*77*	*82*	*82*
Crude fibre	Calc.	28	28	28	28	30	30	31	31
	Anal.	*28*	*24*	*27*	*28*	*28*	*28*	*30*	*29*
Starch	Calc.	335	335	378	378	375	375	376	376
	Anal.	*354*	*357*	*413*	*409*	*409*	*409*	*413*	*416*
Calcium	Calc.	6.6	12.1	5.7	10.3	5.1	9.0	4.6	8.6
	Anal.	*7.7*	*13.4*	*6.8*	*12.0*	*5.9*	*9.8*	*5.4*	*9.3*
Phosphorus	Calc.	5.6	8.5	5.0	7.5	4.7	6.8	4.5	6.6
	Anal.	*6.4*	*9.3*	*5.9*	*8.6*	*5.1*	*7.2*	*5.1*	*7.1*
Sodium	Calc.	1.5	1.5	1.3	1.3	1.3	1.3	1.3	1.3
	Anal.	*1.5*	*1.4*	*1.3*	*1.3*	*1.3*	*1.3*	*1.4*	*1.3*
Potassium	Calc.	9.8	9.8	8.6	8.6	8.5	8.5	8.3	8.3
	Anal.	*9.7*	*9.7*	*8.7*	*8.5*	*8.3*	*8.5*	*8.1*	*8.0*
Chloride	Calc.	1.7	1.7	1.6	1.6	1.6	1.6	1.6	1.6
	Anal.	*1.8*	*1.7*	*1.6*	*1.6*	*1.6*	*1.6*	*1.6*	*1.6*

[1]) Most of the analysis are based on one feed sample, which is analysed in duplo. Values of analysed contents of dry matter, ash, Ca, and P are based on two feed samples analysed in duplo.

Calculated and analysed nutrient contents corresponded well for ash, crude protein, crude fibre, sodium, potassium and chloride. Averaged analysed dry matter content was 22 g/kg higher compared to the calculated contents, which might be related to the high ambient temperature (± 28^0C) during production, as well as to the low moisture content of the heat treated wheat. Analysed Ca and P contents were on average 1.0 and 0.7 g/kg higher than calculated, respectively. Differences were relatively large in phase 1 and 2 diets, and relatively small in phase 3 and 4 diets. This might be related to an overestimation of the Ca and P contents of maize or soybean meal, or an underestimation of the Ca and P contents of wheat. The differences between calculated and analysed Ca:P ratios were very small and in most cases negligible.

3.3 Phosphorus and calcium absorbability

In Table 5, the ileal / pre-caecal P absorption coefficient (%) is provided per treatment and per period.

Table 5

Ileal / pre-caecal Phosphorus absorption coefficient (%) of broilers per treatment and per period.

Absorbable P level (% of CVB)	Phase 1 d0-10	Phase 2 d11-21	Phase 3 d22-30	Phase 4 d31-39
75.0	59.7[ab]	60.0[a]	51.7	53.7
87.5	56.1[b]	56.6[ab]	51.3	57.3
100.0	54.4[bc]	55.3[b]	49.8	53.8
112.5	50.2[c]	52.9[b]	48.3	52.1
125.0	50.1[c]	55.1[b]	51.6	50.2
137.5	61.3[a]	55.5[b]	51.0	53.3
SE	2.33	1.86	1.43	2.70
P-value	< 0.001	0.026	0.187	0.222

[a,b,c] Means in the same column with different superscripts differ significantly (*P* < 0.05)

In phase 1, the P absorption coefficient decreased from 59.7% in birds fed the 75% aP-diet to 50.1% in birds fed the 125% aP-diet. The P absorption coefficient of the birds fed the 137.5% aP-diet was higher than all other treatments, except for birds fed the 75% aP-diet.
In phase 2, the P absorption coefficient of the birds fed the 75% aP-diet was increased compared to the birds fed the 100% or higher aP-diets, whereas the P absorption coefficient of the birds fed the 87.5% aP-diet was in between.
The P absorption coefficient was not affected by dietary aP-level in phase 3 and phase 4.
Table 6 provides the analysed dietary aP contents per treatment and per period.

Table 6

Absorbable phosphorus content (g/kg) based on analysed P content and determined P absorbability per period and per treatment.

Phase	Phase 1 d0-10		Phase 2 d11-21		Phase 3 d22-30		Phase 4 d31-39	
Absorbable P level (% of CVB)	Calculated (g/kg)	Analysed (g/kg)	Calculated (g/kg)	Analysed (g/kg)	Calculated (g/kg)	Analysed (g/kg)	Calculated (g/kg)	Analysed (g/kg)
75.0	3.0	3.8[b]	2.6	3.6[d]	2.3	2.6[e]	2.1	2.7[c]
87.5	3.5	3.8[b]	3.0	3.7[cd]	2.6	2.8[d]	2.5	3.1[b]
100.0	4.0	3.9[b]	3.4	3.9[cd]	3.0	3.0[c]	2.8	3.2[b]
112.5	4.5	3.8[b]	3.8	4.0[c]	3.4	3.1[c]	3.2	3.3[b]
125.0	5.0	4.0[b]	4.3	4.4[b]	3.8	3.5[b]	3.5	3.3[b]
137.5	5.5	5.4[a]	4.7	4.8[a]	4.1	3.7[a]	3.9	3.8[a]
SE		0.19		0.13		0.09		0.15
P-value		< 0.001		< 0.001		< 0.001		< 0.001

[a,b,c,d] Means in the same column with different superscripts differ significantly (*P* < 0.05)

The analysed dietary aP-content increased with increasing aP supply. In all phases, the calculated aP content was underestimated at aP-levels below 100% of the CVB requirements. In phases 1, 2 and 4, the calculated and analysed aP contents at the 137.5% aP-level were in the same range, but this was not the case for phase 3.
Table 7 provides the ileal calcium absorption coefficients per treatment and per period.

Table 7

Ileal calcium absorption coefficient (%) per treatment and per period.

Absorbable P level (% of CVB)	Phase 1 d0-10	Phase 2 d11-21	Phase 3 d22-30	Phase 4 d31-39
75.0	58.9[a]	54.4[a]	47.0[a]	49.6[ab]
87.5	53.6[ab]	48.1[ab]	45.7[ab]	53.7[a]
100.0	53.5[ab]	44.4[bc]	40.1[bc]	49.1[b]
112.5	35.9[c]	39.4[d]	39.4[c]	40.2[c]
125.0	26.6[d]	39.9[cd]	36.6[c]	41.7[c]
137.5	50.6[b]	38.1[d]	36.1[c]	40.4[c]
SE	3.77	2.39	2.26	2.10
P-value	< 0.001	<0.001	<0.001	<0.001

[a,b,c,d] Means in the same column with different superscripts differ significantly ($P < 0.05$)

Ileal Ca absorption levels decreased with increasing dietary P (and Ca) supply. This, however, was not valid for the 137.5% aP diet in phase 1, which showed an increase in Ca absorption after a decreasing trend in the range of the 75% to 125% aP diets.

In Table 8, the analysed absorbable calcium contents (aCa; g/kg) are shown per period and per treatment.

Table 8

Absorbable calcium content (g/kg) based on analysed Ca content and determined Ca absorbability per period and per treatment.

Absorbable P level (% of CVB)	Phase 1 d0-10	Phase 2 d11-21	Phase 3 d22-30	Phase 4 d31-39
75.0	4.5[bc]	3.7[b]	2.9[c]	2.7[d]
87.5	4.7[bc]	3.8[b]	3.0[bc]	3.3[bc]
100.0	5.3[b]	3.9[b]	3.0[bc]	3.4[ab]
112.5	4.0[cd]	3.9[b]	3.2[abc]	3.1[c]
125.0	3.3[d]	4.4[a]	3.3[ab]	3.5[ab]
137.5	6.8[a]	4.6[a]	3.5[a]	3.7[a]
SE	0.39	0.24	0.19	0.16
P-value	< 0.001	0.006	0.009	< 0.001

[a,b,c,d] Means in the same column with different superscripts differ significantly ($P < 0.05$)

In phase 1, the aCa content was highest (6.8 g/kg) in birds fed the 137.5% aP-diet and lowest (3.3 g/kg) in birds fed the 125% aP-diet. The aCa content in the birds fed the 75% to 112.5% aP-diets varied in the range of 4.0 g/kg (112.5% aP diet) to 5.3 g/kg (100% aP-diet).

In phase 2, the aCa contents in birds fed the 125% and 137.5% aP-diets were increased compared to the aCa contents in birds fed the other dietary treatments.

In phase 3, the aCa content gradually increased from 2.9 g/kg in birds fed the 75% aP-diet to 3.5 g/kg in birds fed the 137.5% aP-diet. Likewise, in phase 4, the aCa content gradually increased from 2.7 g/kg in birds fed the 75% aP-diet to 3.7 g/kg in birds fed the 137.5% aP-diet.

3.4 Phytate degradation

In Table 9, phytate degradation (%) and the amount of released phosphorus, due to phytate degradation, expressed in g/kg diet at d21 and d38 is provided.

Table 9

Phytate degradability (%) and the amount of released phosphorus (g/kg diet) at d21 and d38.

	Day 21		Day 38	
Absorbable P level (% of CVB)	Phytate degradability (%)	Released phosphorus (g/kg diet)	Phytate degradability (%)	Released phosphorus (g/kg diet)
75.0	35.4[a]	0.81[a]	43.8[ab]	1.10[a]
87.5	23.4[ab]	0.54[b]	46.8[a]	1.17[a]
100.0	17.4[bc]	0.40[bc]	36.8[bc]	0.92[a]
112.5	8.9[d]	0.20[cd]	24.3[cd]	0.61[b]
125.0	11.7[cd]	0.27[cd]	17.7[d]	0.44[b]
137.5	7.5[d]	0.17[d]	19.7[d]	0.49[b]
SE	4.44	0.10	3.97	0.10
P-value	< 0.001	< 0.001	< 0.001	< 0.001

[a,b,c,d] Means in the same column with different superscripts differ significantly ($P < 0.05$)

At d21, phytate degradability decreased with increasing aP-content from 35.4% in birds fed the 75% aP-diet to 7.5% in birds fed the 137.5% aP-diet. The corresponding amount of released P decreased from 0.81 g/kg in birds fed the 75% aP diet to 0.17 g/kg in birds fed the 137.5% aP-diet.
At d38, phytate degradability decreased with increasing aP-content from 43.8% in birds fed the 75% aP-diet to 17.7% in birds fed the 125% aP-diet. The corresponding amount of released P decreased from 1.10 g/kg in birds fed the 75% aP diet to 0.44 g/kg in birds fed the 125% aP-diet.

3.5 Performance results

The technical performance per treatment of the broilers in the different periods are presented in Table 9 to 12.

3.5.1 Phase 1 (d0-d10)

Table 10 showed the data per treatment for phase 1 (0 to 10 d). Birds receiving the 137.5% aP-diet during phase 1 showed over this period the highest body weight gain (27.2 g/d) and body weight at day 10 (316 g). Birds receiving the 75.0 or 87.5% aP-diets had the lowest body weight gain (25.5 and 26.1 g/d, respectively), and body weight (299 and 304 g, respectively). Birds receiving the 100.0, 112.5, and 125.0% aP-diets showed no differences compared to the 137.5% aP-diet. In contrast, birds receiving the 137.5% aP-diet showed a higher body weight and body weight gain compared to the birds fed the 75.0 and 87.5% aP-diets. Feed conversion ratio was reduced for the birds fed the 125.0 and 137.5% aP-diets, compared to the birds fed the 75.0% aP-diet. Feed intake, water intake, water/feed-ratio, and mortality showed no significant differences.

Table 10

Body weight (BW), body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), water intake (WI), water/feed-ratio (W/F) and mortality (Mort) per treatment for phase 1 (d0-10).

Absorbable P level (% of CVB)	BW (g)	BWG (g/d)	FI (g/d)	FCR (g/g)	WI (g/d)	W/F (g/g)	Mort (%)
75.0	299[c]	25.5[c]	32.9	1.29[b]	63.1	1.92	2.0
87.5	304[bc]	26.1[bc]	32.6	1.25[ab]	63.1	1.93	1.2
100.0	310[ab]	26.5[ab]	33.3	1.25[ab]	64.2	1.93	2.3
112.5	309[ab]	26.6[ab]	33.3	1.25[ab]	63.3	1.90	1.0
125.0	311[ab]	26.8[ab]	33.0	1.23[a]	64.2	1.94	1.9
137.5	316[a]	27.2[a]	33.3	1.22[a]	65.6	1.97	1.3
SEM	2.4	0.2	0.2	0.01	0.78	0.02	0.3
P-value linear	<0.001	<0.001	0.074	<0.001	0.733	0.912	0.326
P-value quadr.	0.383	0.333	0.489	0.547	0.507	0.395	0.938

[a,b,c] Means in the same column with different superscripts differ significantly ($P < 0.05$)

The response curves, as well as the broken-stick model fit for BWG and FCR, based on the analysed and calculated dietary aP level (g/kg) are presented in Figure 1. According to the broken-stick models, the aP requirement for maximal BWG during phase 1 was estimated to be 4.0 (± 0.03) g/kg (R^2 = 0.83) and 5.4 (± 0.20) g/kg (R^2 = 0.88) analysed and calculated dietary aP, respectively. The aP requirement for lowest FCR during phase 1 was estimated to be 4.1 (± 0.11) g/kg (R^2 = 0.37) and 5.4 (± 0.70) g/kg (R^2 = 0.32) analysed and calculated dietary aP, respectively.

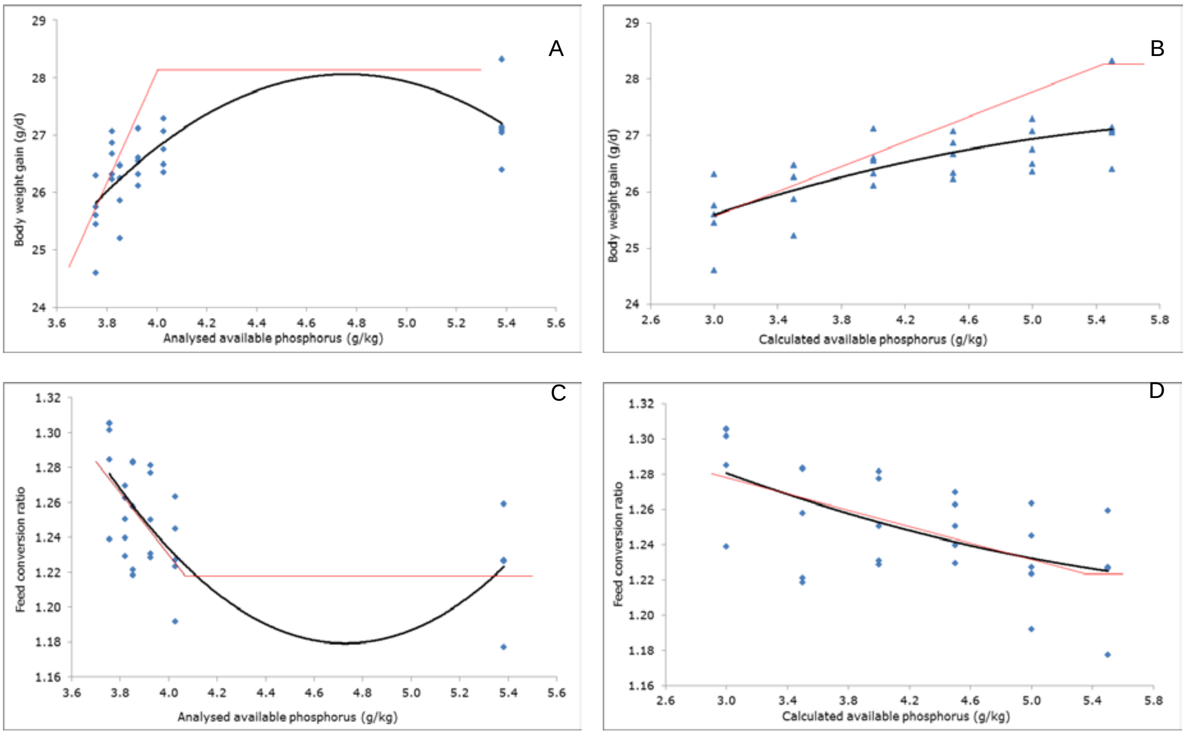

Figure 1 *Relationship between analysed and calculated dietary absorbable phosphorus content on body weight gain(g/d) (plot A, B) and feed conversion ratio (plot C, D) during phase 1; each dot represents one replicate pen.*

3.5.2 Phase 2 (d11-d21)

Table 11 showed the data per treatment for phase 2 (11 to 21 d). Birds fed the 125.0 and 137.5% aP-diet showed higher responses in BW (1023 g) and BWG (64.6 g/d) compared to birds fed the 75.0 and 87.5% aP-diets (984 g and 62 g/d), whereas the BW and BWG of the other treatments were in between. Birds fed the 125.0% aP-diet showed a better FCR (1.33) compared to birds fed the 75.0 and 87.5% aP-diets (1.37), whereas the FCR levels of the other treatments were in between. Water intake and water to feed ratio increased with increasing dietary aP content, especially in the range of 100 to 137.5% aP. In the range of 75 to 112.5% aP feed intake increased with increasing aP content, overall following a quadratic trend (*P*=0.104). The water to feed ratio showed a quadratic response the P supply. The ratio decreased in the range of 75 to 100% aP, where after it increased to 137.5% aP. Mortality was not affected by dietary treatments.

Table 11
Body weight (BW), body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), water intake (WI), water/feed-ratio (W/F) and mortality (Mort) per treatment for phase 2 (d11-21).

Absorbable P level (% of CVB)	BW (g)	BWG (g/d)	FI (g/d)	FCR (g/g)	WI (g/d)	W/F (g/g)	Mort (%)
75.0	984^{b}	62.2^{b}	84.6^{b}	1.36^{ab}	151.9^{c}	1.80^{b}	2.3
87.5	984^{b}	61.8^{b}	85.2^{ab}	1.38^{b}	152.0^{c}	1.78^{b}	2.7
100.0	1003^{ab}	63.0^{ab}	85.8^{ab}	1.36^{ab}	153.9^{bc}	1.79^{b}	2.4
112.5	1010^{a}	63.7^{ab}	86.0^{a}	1.35^{ab}	156.8^{abc}	1.82^{ab}	1.9
125.0	1023^{a}	64.8^{a}	86.0^{a}	1.33^{a}	157.6^{ab}	1.83^{ab}	2.4
137.5	1023^{a}	64.4^{a}	86.0^{a}	1.34^{ab}	161.7^{a}	1.88^{a}	2.1
SEM	6.4	0.5	0.4	0.01	1.3	0.01	0.8
P-value linear	<.0001	<0.001	0.005	0.002	<.0001	0.001	0.267
P-value quadr.	0.463	0.806	0.104	0.478	0.225	0.042	0.888

^{a,b,c} Means in the same column with different superscripts differ significantly ($P < 0.05$)

The response curves, as well as the broken-stick model fit for BWG and FCR, based on the analysed and calculated dietary aP level (g/kg) are presented in Figure 2. According to the broken-stick models, the aP requirement for maximal BWG during phase 2 was estimated to be 4.2 (± 0.20) g/kg (R^2 = 0.47) and 4.3 (± 0.06) g/kg (R^2 = 0.45) analysed and calculated dietary aP, respectively. The aP requirement for lowest FCR during phase 2 was estimated to be 4.4 (± 0.06) g/kg (R^2 = 0.25) analysed dietary aP. No broken stick model could be fitted for the response of calculated aP content on FCR.

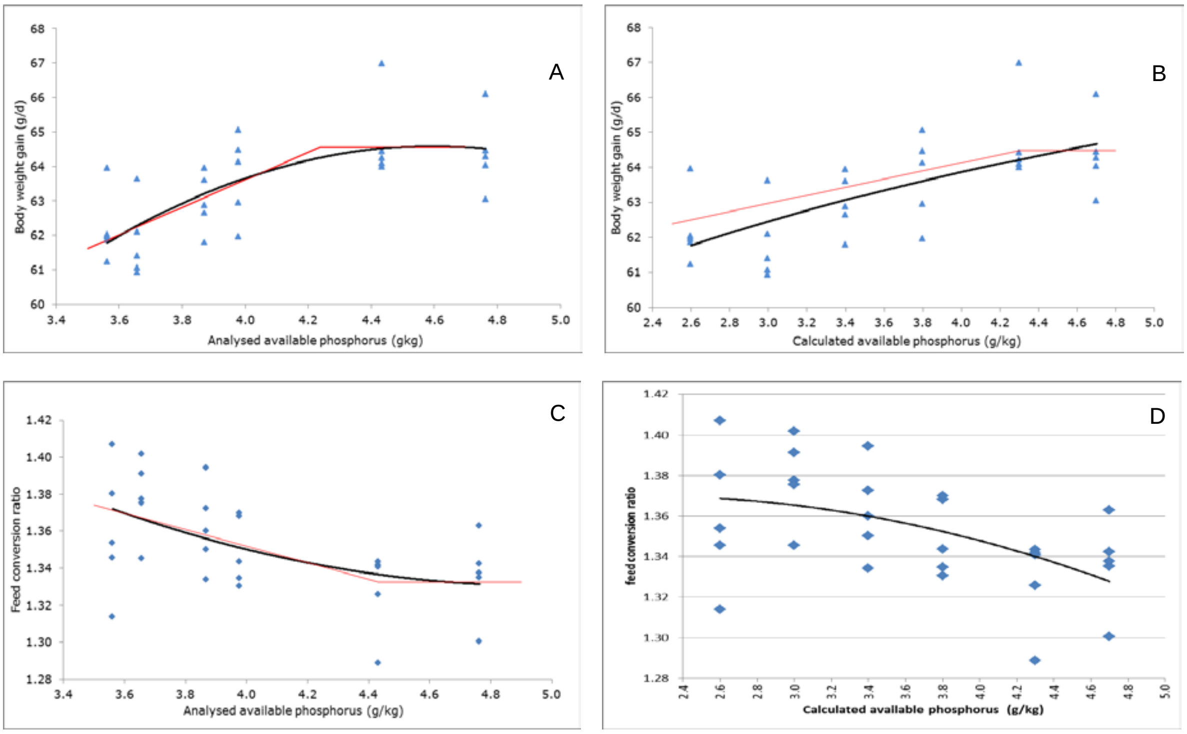

Figure 2 *Relationship between analysed and calculated dietary absorbable phosphorus content on body weight gain(g/d) (plot A, B) and feed conversion ratio (plot C) during phase 2; each dot represents one replicate pen.*

3.5.3 Phase 3 (d22-d30)

Table 12 showed the data per treatment for phase 3 (22 to 30 d). In this period, BWG was not affected by dietary treatments. BW, however, showed in the range of 75% to 100% aP an increased trend with increasing aP-content. Differences in BW already developed in phase 1 and 2 periods, and maintained in phase 3. Feed intake was reduced in the birds fed the 75 and 87.5% aP-diets, compared to the other treatments. WI and W/F ratio showed a quadratic response on aP content. WI and W/F ratio decreased with increased aP content in the range of 75 to 112.5% aP, where after values increased up to 137.5% aP. FCR, and mortality were not affected by the treatments.

Table 12
Body weight (BW), body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), water intake (WI), water/feed-ratio (W/F) and mortality (Mort) per treatment for phase 3 (d22-30).

Absorbable P level (% of CVB)	BW (g)	BWG (g/d)	FI (g/d)	FCR (g/g)	WI (g/d)	W/F (g/g)	Mort (%)
75.0	1814[c]	92.2	150.2[b]	1.63	235.4	1.57	2.1
87.5	1816[bc]	92.4	149.7[b]	1.62	230.2	1.54	1.9
100.0	1851[abc]	94.0	151.9[a]	1.62	231.3	1.52	1.7
112.5	1858[abc]	94.2	151.8[a]	1.61	230.6	1.52	2.0
125.0	1862[ab]	93.1	151.6[a]	1.63	231.4	1.53	1.5
137.5	1872[a]	94.2	151.7[a]	1.61	236.0	1.56	1.8
SEM	11.8	1.0	0.5	0.02	2.8	0.02	0.7
P-value linear	0.001	0.102	0.003	0.516	0.687	0.363	0.064
P-value quadr.	0.298	0.326	0.094	0.644	0.014	0.002	0.461

[a,b,c] Means in the same column with different superscripts differ significantly ($P < 0.05$)

Because of the absence of a response of BWG and FCR to aP content, no response curves and broken-stick models could be plotted for these parameters.

3.5.4 Phase 4 (d31-39)

Table 13 showed the data per treatment for phase 4 (31 to 39 d). Body weight, body weight gain, feed intake, FCR, and water to feed ratio were significantly affected by treatments. BW increased with increasing dietary aP content. The response of BW to aP content showed both a linear and quadratic pattern. BWG of the birds fed the 75% aP-diet was reduced compared to birds fed the 112.5% or 125% aP-diets, whereas the other treatments were in between. Feed intake gradually increased with increasing aP contents. Feed intake levels of the birds fed the 75% and 87.5% aP diets was reduced compared to the birds fed the 137.5% diets, whereas the feed intake level of the other treatments were in between. The response of FCR to aP content showed a quadratic pattern. FCR of birds fed the 75% or the 137.5% aP diets was increased compared to the FCR of birds fed the 87.5% aP diet, whereas the FCR of the other treatments was in between. The W/F ratio gradually decreased with increasing dietary aP contents. The W/F ratio of the birds fed the 75% aP-diet was increased compared to the birds fed the 137.5% diet, whereas the W/F ratio of the other treatments were in between.

Table 13

Body weight (BW), body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), water intake (WI), water/feed-ratio (W/F) and mortality (Mort) per treatment for phase 4 (d31-39).

Absorbable P level (% of CVB)	BW (g)	BWG (g/d)	FI (g/d)	FCR (g/g)	WI (g/d)	W/F (g/g)	Mort (%)
75.0	2542[c]	82.1[b]	161.8[b]	1.98[a]	302.7	1.85[a]	1.8
87.5	2605[bc]	87.9[ab]	165.1[b]	1.88[c]	300.4	1.82[ab]	1.7
100.0	2655[ab]	89.1[ab]	169.1[ab]	1.90[bc]	298.5	1.77[ab]	1.9
112.5	2647[ab]	90.7[a]	170.1[ab]	1.89[bc]	300.2	1.77[ab]	2.0
125.0	2668[ab]	89.8[a]	168.2[ab]	1.88[c]	295.6	1.76[ab]	1.9
137.5	2674[a]	89.1[ab]	175.3[a]	1.97[ab]	304.1	1.74[b]	2.0
SEM	18.3	1.9	2.5	0.03	3.6	0.02	0.7
P-value linear	<0.001	0.011	0.002	0.403	0.758	<0.001	0.199
P-value quadr.	0.007	0.069	0.845	0.059	0.130	0.252	0.971

[a,b,c] Means in the same column with different superscripts differ significantly ($P < 0.05$)

The response curves, as well as the broken-stick model fit for BWG and FCR, based on the analysed and calculated dietary aP level (g/kg) are presented in Figure 3. According to the broken-stick models, the aP requirement for maximal BWG during phase 4 was estimated to be 3.1 (± 0.07) g/kg (R^2 = 0.30) and 2.6 (± 0.13) g/kg (R^2 = 0.30) analysed and calculated dietary aP, respectively. The aP requirement for lowest FCR during phase 4 was estimated to be 3.1 (SE not provided, because of an unsuccessful optimization: a parameter has gone out of bounds) g/kg (R^2 = 0.17) and 2.5 (SE not provided, because of an unsuccessful optimization: a parameter has gone out of bounds) g/kg (R^2 = 0.17) analysed and calculated dietary aP, respectively.

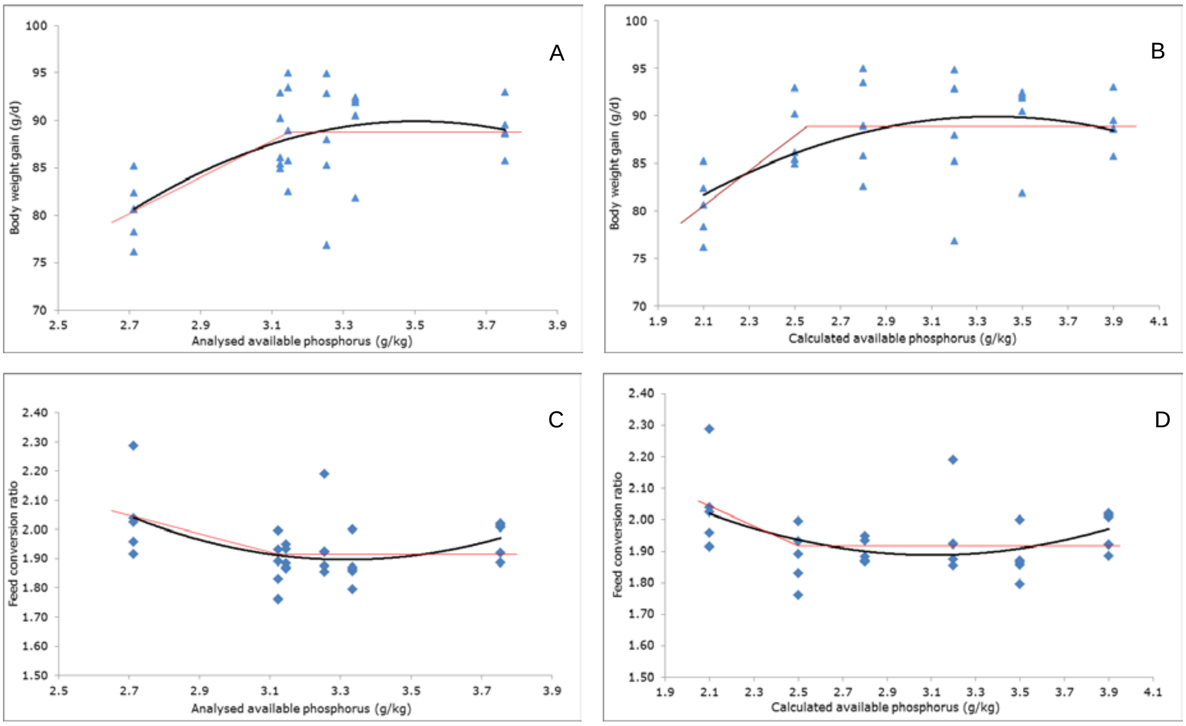

Figure 3 *Effect of analysed and calculated dietary absorbable phosphorus content on body weight gain(g/d) (plot A, B) and feed conversion ratio (plot C, D) during phase 4; each dot represents one replicate pen.*

To summarize, this study showed that for realizing maximal performance, in phase 1 (d1-d10) 5.4 g calculated aP per kg of feed was required, in phase 2 (d11-d21) 4.3 g calculated aP per kg of feed, and in phase 4 (d31-d38) 2.6 g calculated aP per kg of feed. In phase 3 (d22-d30), performance of the broilers was not affected by the calculated aP content.

On the basis of analysed aP, this study showed that for realizing maximal performance, in phase 1 (d1-d10) 4.0 g aP per kg of feed was required, in phase 2 (d11-d21) 4.2 g aP per kg of feed, and in phase 4 (d31-d38) 3.1 g aP per kg of feed. In phase 3 (d22-d30), performance of the broilers was not affected by the analysed aP content.

3.6 Footpad lesions, feather quality, and litter quality

The dietary treatments did not affect footpad lesions, feather quality, and litter quality in the current experiment.

3.7 Tibia characteristics

The tibia characteristics per treatment of the broilers in the different periods are presented in Table 9 to 12.

3.7.1 Phase 1 (d0-10)

Table 14 showed the tibia characteristics per treatment for phase 1 (0 to 10 d). Tibia weight and tibia ash weight were significantly affected by dietary treatment. Values varied among aP levels, although no linear or quadratic relationships were observed. Breaking strength of tibia tended (P=0.085) to show a quadratic response. The breaking strength decreased with increasing aP content over the range of 75 to 125%, where after breaking strength increased at the 137.5% aP level. Tibia ash content tended (P=0.053) to increase linearly with increasing dietary aP content.

Table 14
Tibia characteristics per treatment for phase 1 (d0-10).

Absorbable P level (% of CVB)	Breaking strength (Nmm)	Tibia diameter (mm)	Tibia weight (g)	Strength / mm tibia (N)	Strength / g tibia (N)	Tibia ash content (g/kg)	Tibia ash weight (g)
75.0	95.2	3.38	8.38^{bc}	28.37	11.34	87.6	0.25^{ab}
87.5	95.5	3.35	8.97^{ab}	28.38	10.64	90.3	0.27^{ab}
100.0	91.3	3.41	9.57^{a}	26.65	9.68	87.5	0.28^{ab}
112.5	71.7	3.26	7.87^{cd}	21.96	9.12	92.1	0.24^{b}
125.0	68.5	3.09	7.36^{d}	22.18	9.38	94.1	0.23^{b}
137.5	96.9	3.38	9.17^{ab}	28.61	10.55	95.2	0.29^{a}
SE	12.33	0.13	0.48	3.32	1.30	4.23	0.014
P-value linear	0.220	0.232	0.370	0.161	0.292	0.053	0.383
P-value quadr.	0.085	0.430	0.589	0.274	0.106	0.690	0.206

^{a,b,c,d} Means in the same column with different superscripts differ significantly (P < 0.05)

3.7.2 Phase 2 (d11-21)0

Table 15 showed the tibia characteristics per treatment for phase 2 (11 to 21 d). Tibia breaking strength tended (P=0.101) to increase linearly with increasing dietary aP content. Likewise, tibia breaking strength expressed per mm tibia section tended (P=0.079) to increase linearly with increasing dietary aP content. Tibia weight and tibia ash weight linearly increased with increasing dietary aP content. tended (P = 0.07) to increase with increasing aP content. Tibia ash content of the birds fed the 75% aP-diet was reduced compared to the birds fed the 112.5% or 137.5% aP-diet, whereas the tibia ash contents of the other treatments were in between.

Table 15

Tibia characteristics per treatment for phase 2 (d11-21).

Absorbable P level (% of CVB)	Breaking strength (Nmm)	Tibia diameter (mm)	Tibia weight (g)	Strength/ mm tibia (N)	Strength/ g tibia (N)	Tibia ash content (g/kg)	Tibia ash weight (g)
75.0	361	5.18	32.0	69.6	11.3	89.7^{b}	0.98^{b}
87.5	382	5.42	33.8	70.5	11.4	95.2^{ab}	1.10^{ab}
100.0	380	5.27	34.7	72.1	11.0	95.9^{ab}	1.13^{ab}
112.5	431	5.39	33.0	79.7	13.2	101.2^{a}	1.12^{ab}
125.0	432	5.23	36.0	82.8	12.1	96.5^{ab}	1.19^{a}
137.5	414	5.27	35.6	78.5	11.7	102.5^{a}	1.24^{a}
SE	45.2	0.15	1.39	8.01	1.53	3.41	0.052
P-value linear	0.101	0.948	0.011	0.079	0.521	0.002	<0.001
P-value quadr.	0.506	0.304	0.776	0.675	0.568	0.327	0.508

[a,b] Means in the same column with different superscripts differ significantly (P < 0.05)

The response curves, as well as the broken-stick model fit for tibia ash content, based on the analysed and calculated dietary aP level (g/kg) are presented in Figure 4. According to the broken-stick models, the aP requirement for maximal tibia ash content during phase 2 was estimated to be 4.0 (± 0.03) g/kg (R^2 = 0.24) and 3.8 (± 0.03) g/kg (R^2 = 0.26) analysed and calculated dietary aP, respectively.

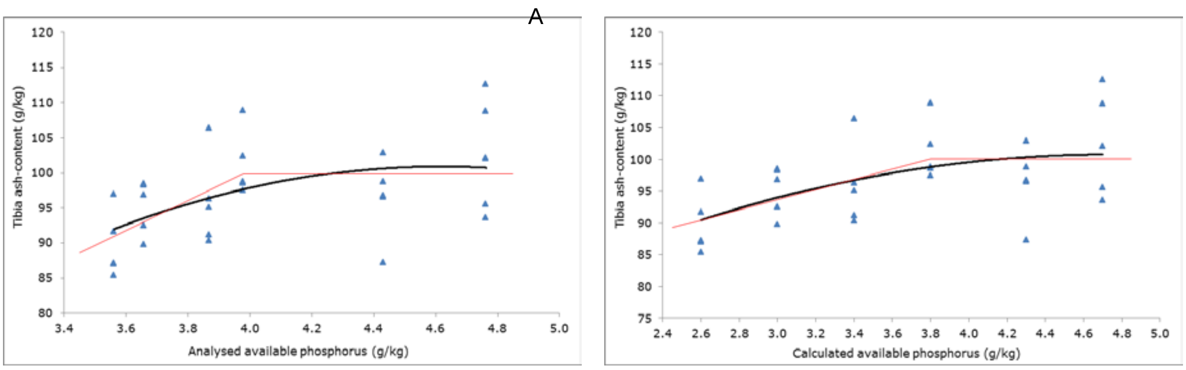

Figure 4 *Effect of analysed and calculated dietary absorbable phosphorus content (g/d) (plot A, B) on tibia ash content during phase 2; each dot represents one replicate pen.*

3.7.3 Phase 3 (d22-30)

Table 16 showed the tibia characteristics per treatment for phase 3 (22 to 30 d). Tibia breaking strength, both absolute and expressed per mm tibia section, tibia weight, tibia ash content and ash weight linearly increased with increasing dietary aP content. Tibia breaking strength, expressed per g tibia, tended (P=0.069) to increase with increasing aP content of the diet. Tibia ash content of birds fed the 125% aP diet was higher compared to the that of the birds fed the 75% or 100% aP diets, where the other treatments were in between.

Table 16

Tibia characteristics per treatment for phase 3 (d22-30).

Absorbable P level (% of CVB)	Breaking strength (Nmm)	Tibia diameter (mm)	Tibia weight (g)	Strength / mm tibia (N)	Strength / g tibia (N)	Tibia ash content (g/kg)	Tibia ash weight (g)
75.0	654	6.89	42.6	76.8	12.3	128.5[b]	1.85[c]
87.5	666	6.70	42.7	99.7	15.6	132.0[ab]	1.91[bc]
100.0	669	6.72	44.3	99.5	15.1	131.2[b]	1.96[abc]
112.5	761	6.93	44.6	109.8	17.0	134.8[ab]	2.00[abc]
125.0	704	6.69	45.7	105.1	15.5	141.2[a]	2.16[a]
137.5	753	6.75	45.2	111.6	16.7	136.6[ab]	2.08[ab]
SE	51.1	0.23	1.49	12.78	2.02	2.86	0.065
P-value linear	0.032	0.726	0.017	0.017	0.069	<0.001	<0.001
P-value quadr.	0.818	0.961	0.625	0.247	0.275	0.661	0.592

[a,b,c] Means in the same column with different superscripts differ significantly ($P < 0.05$)

The response curves, as well as the broken-stick model fit for tibia ash content, based on the analysed and calculated dietary aP level (g/kg) are presented in Figure 5. According to the broken-stick models, the aP requirement for maximal tibia ash content during phase 3 was estimated to be 3.5 (± 0.15) g/kg (R^2 = 0.39) and 3.8 (± 0.03) g/kg (R^2 = 0.37) analysed and calculated dietary aP, respectively.

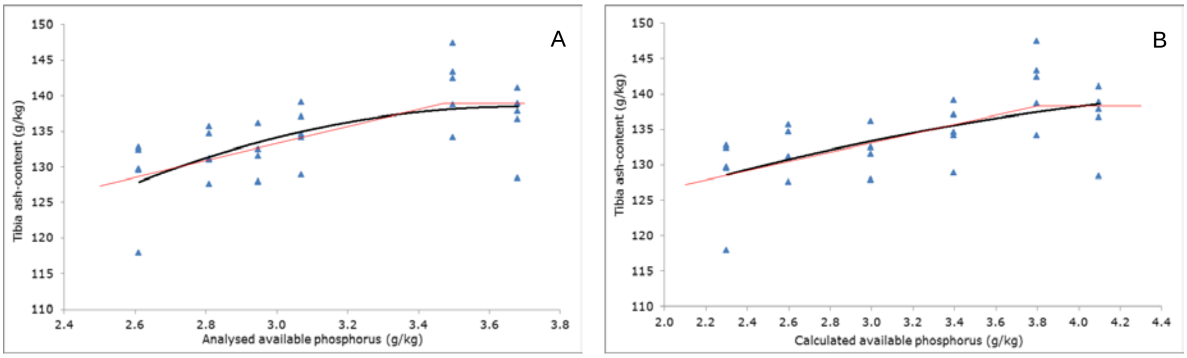

Figure 5 *Effect of analysed and calculated dietary absorbable phosphorus content (g/d) (plot A, B) on tibia ash content during phase 3; each dot represents one replicate pen.*

3.7.4 Phase 4 (d31-39)

Table 17 showed the tibia characteristics per treatment for phase 4 (31 to 39 d). All observed parameters showed a linear response on increasing aP content of the diet. Tibia breaking strength, both absolute and expressed per mm bone section, and tibia ash content of birds in phase 4 increased with increasing dietary aP content over the range of 75% to 125%, whereas values did not further increase in birds fed the 137.5% aP-diet. Tibia breaking strength, expressed per mm bone section and per g tibia, tended to have a quadratic relationship with dietary aP content.

Table 17
Tibia characteristics per treatment for phase 4 (d31-39).

Absorbable P level (% of CVB)	Breaking strength (Nmm)	Tibia diameter (mm)	Tibia weight (g)	Strength / mm tibia (N)	Strength / g tibia (N)	Tibia ash content (g/kg)	Tibia ash weight (g)
75.0	652^{b}	7.66	20.76^{b}	85.7^{b}	10.69	124.2^{b}	2.58^{c}
87.5	788^{ab}	7.55	21.85^{b}	104.3^{ab}	12.18	131.2^{ab}	2.87^{bc}
100.0	819^{ab}	7.68	20.94^{b}	106.5^{ab}	13.25	134.1^{ab}	2.81^{bc}
112.5	806^{ab}	7.79	21.75^{b}	103.5^{ab}	12.59	135.3^{ab}	2.94^{abc}
125.0	982^{a}	7.95	22.11^{ab}	123.6^{a}	14.64	140.6^{a}	3.10^{ab}
137.5	875^{ab}	8.29	23.99^{a}	106.2^{ab}	12.55	139.6^{a}	3.35^{a}
SE	75.4	0.30	1.789	9.76	1.17	3.69	0.125
P-value linear	0.001	0.020	<0.001	0.014	0.035	<0.001	<0.001
P-value quadr.	0.171	0.228	0.113	0.079	0.069	0.205	0.478

[a,b,c] Means in the same column with different superscripts differ significantly ($P < 0.05$)

$ Means a tendency to a difference ($0.05<P<0.10$)

The response curves, as well as the broken-stick model fit for tibia breaking strength per mm bone and for tibia ash content, based on the analysed and calculated dietary aP level (g/kg) are presented in Figure 6.

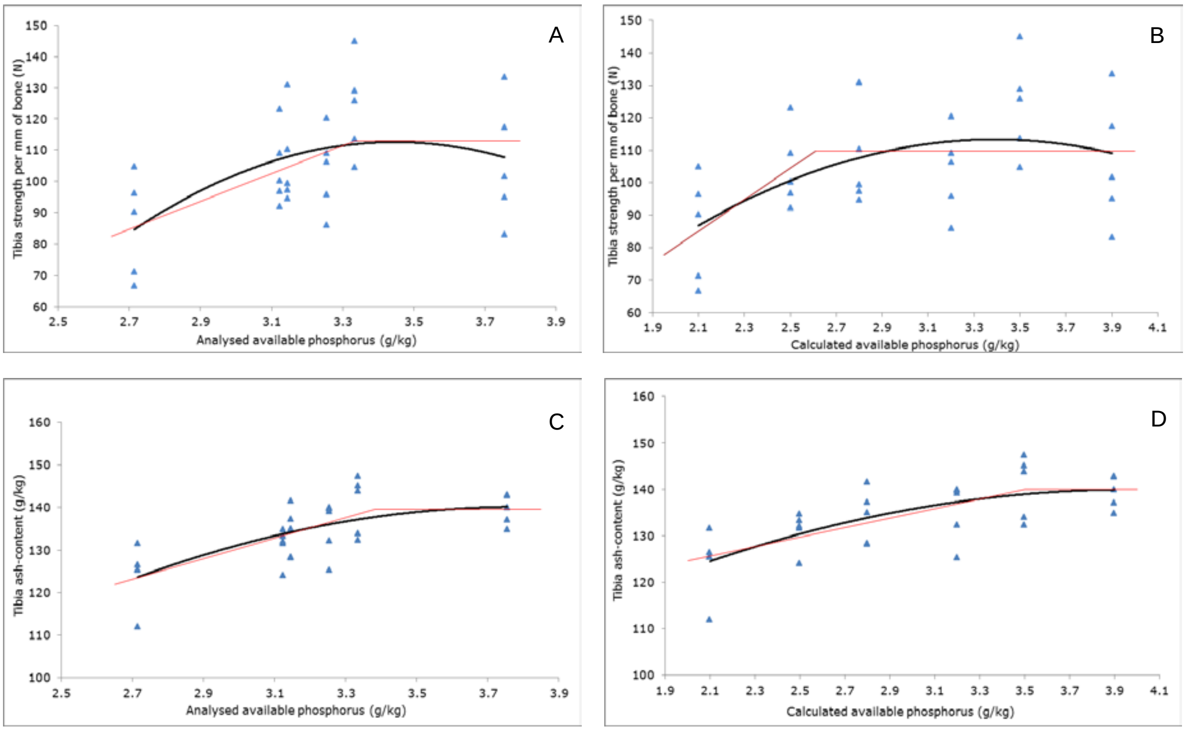

Figure 6 *Effect of analysed and calculated dietary absorbable phosphorus content (g/d) on tibia breaking strength per mm bone (plot A, B) and tibia ash content (plot C, D) during phase 4; each dot represents one replicate pen.*

According to the broken-stick models, the aP requirement for maximal tibia breaking strength per mm bone during phase 4 was estimated to be 3.2 (± 0.16) g/kg (R^2 = 0.20) and 2.6 (± 0.22) g/kg (R^2 = 0.20) analysed and calculated dietary aP, respectively. The aP requirement for maximal tibia ash content during phase 4 was estimated to be 3.4 (± 0.13) g/kg (R^2 = 0.45) and 3.5 (± 0.09) g/kg (R^2 = 0.46) analysed and calculated dietary aP, respectively.

3.8 Phosphorus and calcium contents in empty body weight

Table 18 shows the P contents in the carcass of the birds, expressed in g per kg empty body weight, determined at d10, d21, d30, and d38.

Table 18

Phosphorus contents (g/kg empty body weight) at d10, 21, 30 and 38.

Absorbable P level (% of CVB)	Day 10 (g/kg)	Day 21 (g/kg)	Day 30 (g/kg)	Day 38 (g/kg)
75.0	4.40	4.42	4.20	3.91
87.5	4.39	4.73	4.52	4.25
100.0	4.29	4.82	4.46	4.13
112.5	4.55	4.68	4.67	4.26
125.0	4.56	4.88	4.49	4.15
137.5	4.83	4.96	4.42	4.44
SE	0.18	0.19	0.25	0.26
P-value linear	0.013	0.014	0.458	0.127
P-value quadr.	0.125	0.563	0.161	0.914

P content in the carcass was not significantly affected by dietary aP-content on Day 30 and 38, whereas at d10 and d21, a linear positive relationship was found between the P content in the carcass and the aP supply. Average P content in the carcass was 4.50, 4.75, 4.48, and 4.19 g/kg at d10, d21, d30, and d38, respectively.
Table 19 showed the Ca contents in the carcasses of the birds, expressed in g per kg empty body weight, determined at d10, d21, d30, and d38.

Table 19

Calcium contents (g/kg empty body weight) at d10, 21, 30 and 38.

Absorbable P level (% of CVB)	Day 10 (g/kg)	Day 21 (g/kg)	Day 30 (g/kg)	Day 38 (g/kg)
75.0	5.26[ab]	5.71[b]	5.49	5.06
87.5	5.46[ab]	6.23[ab]	6.23	5.69
100.0	5.12[b]	6.33[ab]	6.08	5.53
112.5	5.61[ab]	6.25[ab]	6.45	5.72
125.0	5.71[ab]	6.70[ab]	6.35	5.60
137.5	6.13[a]	6.83[a]	5.59	6.05
SE	0.29	0.30	0.33	0.44
P-value linear	0.004	<0.001	0.626	0.087
P-value quadr.	0.146	0.734	0.024	0.810

[a,b] Means in the same column with different superscripts differ significantly ($P < 0.05$)

At d10, Ca content in the carcass increased with increasing dietary aP-content, from 5.26 g/kg in birds fed the 75% aP-diet to 6.13 g/kg in birds fed the 137.5% aP diet. Likewise, at d21, Ca content in the carcass increased with increasing dietary aP-content, from 5.71 g/kg in birds fed the 75% aP-diet to 6.83 g/kg in birds fed the 137.5% aP diet. At d30, a quadratic relationship was found between the dietary aP content and the Ca content in the carcass. Ca carcass content was not affected by dietary aP-content at 38. Average Ca content in the carcass amounted 6.03 and 5.61 g/kg at d30 and d38, respectively.

4 Discussion

The aim of this study was to obtain insight in the absorbable phosphorus requirements of modern commercial broilers for four different periods: phase 1 (from d1-10) phase 2 (from d11-20), phase 3 (from d21-30) and phase 4 (from d31-38). The estimated requirements were based on maximisation of performance (BWG, FCR), tibia breaking strength and bone mineralisation.
The results of the current study were compared with five broiler studies that were published in the literature from 2001 onwards. Runho et al. (2001) studied the aP requirement of broilers from d1-21 of age. Venäläinen et al. (2006) investigated the aP requirement of broilers during the starter period (d1-14) and the finisher period (d15-36). The availability of P in wheat, barley, soybean meal and monocalcium phosphate used in their calculations was 35, 47, 42 and 80% of the analysed total P contents, respectively. Dhandu and Angel (2003) focussed on the non-phytate phosphorus (nPP) requirement of broilers in the finisher phase (d32-49). The nPP-content was calculated as the difference between the analysed dietary total P and phytate P concentrations. Mello et al. (2012) determined the aP requirements of broilers in the periods d1-10, d11-21, d22-33, and d34-38. In this study, the aP content of the feed ingredients was calculated as 33% of total P. Finally, Létourneau-Montminy et al. (2010) performed a meta-analysis to predict the response of 21-day-old broilers to dietary nPP. The nPP-content was calculated as the difference between the estimated dietary total P and phytate P concentrations, based on the table of feedstuffs composition (Sauvant et al., 2004).

4.1 Comparison between calculated and analysed absorbable phosphorus contents

The experimental diets were optimised for aP, based on the tabulated CVB (2011) aP values for feed ingredients. In Figure 7, the calculated (red line) and analysed (blue line) aP contents of the current study are provided for birds dissected at d10, 21, 30 and 38.

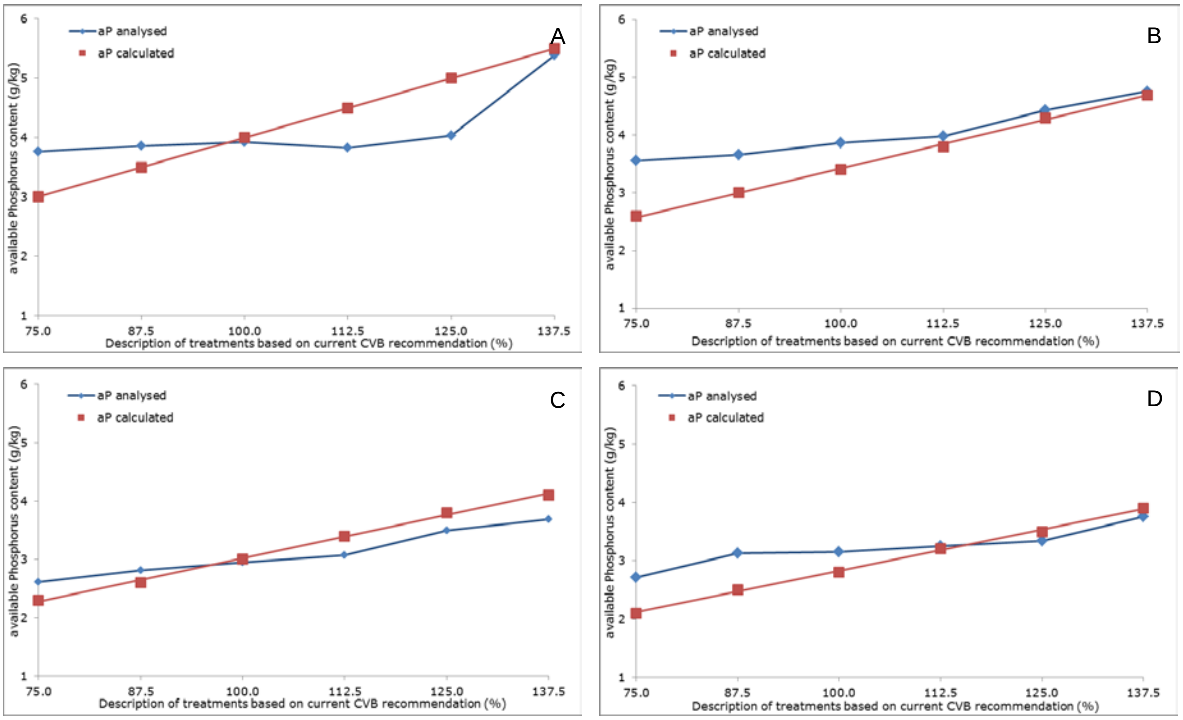

Figure 7 *Comparison between calculated and analysed absorbable phosphorus contents of the current study for birds dissected at d10 (A), 21 (B), 30 (C), and 38 (D).*

Because of higher analysed total dietary P contents than calculated (on average + 0.7 g/kg), it was expected that analysed aP contents in all phases and for all aP levels would be slightly higher than calculated. From this figure, however, it can be concluded that only for broilers at d21, 30 and 38, calculated and analysed aP values were reasonably well in the range of 112.5 to 137.5% of the CVB (2012) requirements. For broilers at 10 d of age, calculated and analysed aP values only agreed at 100 and 137.5% of the CVB (2012) requirements. Interestingly, for the broilers at 21, 30 and 38 d of age, the different pictures all showed a similar pattern, namely that the slopes of the calculated and analyses lines were comparable between the 112.5 and 137.5% aP levels. Similar slopes indicated that P supplementation in this aP range resulted in a similar increase of available P, according to both the calculated and analysed aP values. The slightly higher values of the analysed aP values in the range of 112.5 to 137.5% aP in the 21d old broilers might be the result of the higher analysed dietary P contents. The slightly lower analysed aP values in the range of 112.5 to 137.5% aP in the 30 and 38d old broilers might indicate that P-absorbability of ingredients for birds of these ages are overestimated.

At aP levels below 112.5%, however, the slopes of the analysed aP lines were significantly lower compared to the calculated aP lines. This means that at lower aP supply levels, the broilers were regulating P absorption, thereby preventing a stage of P deficiency. Deviations between the calculated and analysed lines started at 125%, 112.5%, 112.5% and 125% of the CVB (2012) requirements for broilers of 10, 21, 30, and 38d of age, respectively. Below these aP supply levels, the broilers were able to increase the level of P absorption to maintain their metabolic P supply. The likely mode of action is that phytate degradation increased with decreasing aP levels, thereby making more P available for absorption, as demonstrated in Table 10. Phytate degradation could be increased with decreasing P supply until a maximum was achieved. This is clear in the graph from the broilers at 38 d of age. Over the range of 87.5% to 125% of the CVB (2012) requirements, the analysed aP content was constant, whereas below the 87.5% aP supply level the slopes of the analysed and calculated lines again were comparable, reflecting a similar decrease in calculated and analysed aP content. This could imply that the P supply became really deficient below an aP content of 87.5%. This is supported by the tibia breaking strength, which was significantly decreased in 38 day-old broilers that were fed the 75% aP-diet.
Based on these observations, it can be proposed that when decreasing the aP content, the starting point of deviation in slopes between the calculated and analysed aP lines can be considered as an indication of the start of marginal aP supply. From this point downwards, the birds increased their P absorption to compensate for the lower dietary P content. At 10, 21 and 30 d of age, the calculated aP levels which ensured maximal BWG, FCR oror tibia ash content are 137.5%, 125% and 125%, respectively, which is just one aP inclusion level higher compared to the starting points of deviation between slopes of the calculated and analysed aP lines.

4.2 aP-requirements based on performance results

In phase 1 of the current study, aP-requirement for maximal BWG and minimal FCR minimal was estimated to be 5.4 g/kg calculated aP. Based on the analysed dietary aP-contents, the aP-requirement for BWG and FCR was estimated to be 4.0 and 4.1 g/kg, respectively. For 1-10d of age, Mello et al. (2012) estimated the calculated aP-requirement for BWG on 4.4 g/kg (quadratic model) and for FCR on 4.8 g/kg (quadratic model).
In phase 2 of the current study, aP-requirement for maximal BWG and minimal FCR minimal was estimated to be 4.3 g/kg and 4.4 g/kg calculated aP, respectively. Based on the analysed dietary aP-contents, the aP-requirement for BWG was estimated to be 4.2 g/kg, which is in line with the estimated aP-requirement based on calculated aP values. For 11-21d of age, Mello et al. (2012) estimated the calculated aP-requirement for BWG on 3.57 g/kg, and for FCR on 4.8 g/kg, by use of a linear response plateau. Venäläinen et al. (2006), who tested in starter diets calculated aP-levels in the range from 4.0 to 5.5 g/kg, did not find any effect of aP-content on BWG of FCR, indicating that the aP-requirement for 1-14 day old broilers in that study was covered by 4.0 g/kg. Runho et al. (2001) recommended for broilers from 1-21 d of age a calculated dietary aP-level of 4.5 g/kg. Létourneau-Montminy et al. (2010) observed in their meta-analysis a significant effect of dietary Ca-level and microbial phytase addition on the response variables. Their estimated amounts of nPP for

maximising the response of feed intake, BWG, FCR and tibia ash concentration in 21-day-old broilers are presented in Table 20.

Table 20
Estimated amounts of nPP for maximising the response of feed intake, BWG, FCR and tibia ash concentration in 21-day-old broilers according to Létourneau-Montminy et al. (2010).

Ca (g/kg diet)	6		8		10	
Phytase (FTU/kg diet)	0	500	0	500	0	500
Feed intake	4.2	3.8	4.4	4.1	4.5	4.3
Body weight gain	4.0	3.7	4.2	3.9	4.4	4.2
FCR	3.6	3.3	3.8	3.7	4.1	4.1
Tibia ash	4.5	3.9	4.8	4.2	5.2	4.5

It can be concluded from this meta-analysis that the estimated amount of nPP required for maximal response is reduced with decreasing dietary Ca-contents, and with supplementation of 500 FTU phytase compared to unsupplemented diets. The Ca content in the current study at an aP-level of 125% of CVB requirement amounted 9.3 g/kg. In the study of Létourneau-Montminy et al. (2010), the estimated nPP contents for maximal BWG and FCR in diets without phytase, and containing 8 or 10 g/kg Ca ranged from 3.8 to 4.4 g/kg, thereby partly covering the aP-requirements of the current study.

In phase 3 of the current study, BWG and FCR were not affected by the dietary aP-level. Feed intake, however, was reduced at aP-levels below 100% of CVB (2012) requirement, which corresponded with a calculated aP-value of 3.0 g/kg. In line with our findings, Mello et al. (2012) could not fit a significant model for the response of aP on BWG and FCR in broilers of 22-33 d of age. For phase 3 broilers, they recommended a calculated aP content of 3.95 g/kg.
In phase 4 of the current study, aP-requirement for maximal BWG and minimal FCR minimal was estimated to be 2.6 and 2.5 g/kg calculated aP, respectively. Based on the analysed dietary aP-contents, the estimated the aP-requirements for both BWG and FCR were 3.1 g/kg. Mello et al. (2012) could not fit a significant model for the response of aP on BWG and FCR in broilers of 34-46 d of age, but recommended for this phase an aP level of 3.19 g/kg. In the study of Dhandu and Angel (2003), BWG and FCR in 32-42 day-old broilers were not affected by dietary nPP-content in the range of 1.5-3.1 g/kg. Likewise, in the study of Venäläinen et al. (2006), BWG and FCR in 15-36 day-old broilers were not affected by dietary aP-content in the range of 3.5 to 5.0 g/kg.
An overview of required (current study) or recommended (other studies) dietary aP levels for maximal BWG and lowest FCR, is shown in Table 21 and 22, respectively.

Table 21

Overview of required (current study) or recommended (other studies) dietary absorbable phosphorus levels (g/kg) for maximal body weight gain of broilers per phase.

<table>
<tr><th>Source

Basis
Period</th><th>Current study

Calculated aP</th><th>Current study

Analysed aP</th><th>(Mello et al., 2012)

Calculated aP</th><th>(Runho et al., 2001)

Calculated aP</th><th>(Letourneau-Montminy et al., 2010)
nPP</th><th>(Venalainen et al., 2006)

Calculated aP</th></tr>
<tr><td>Phase 1 (D0-10)</td><td>5.4 (± 0.20)</td><td>4.0 (± 0.03)</td><td>4.4</td><td rowspan="2">4.5</td><td rowspan="2">3.7-4.4[2]</td><td rowspan="2">4.0[3]</td></tr>
<tr><td>Phase 2 (D11-21)</td><td>4.3 (± 0.06)</td><td>4.2 (± 0.20)</td><td>3.6[1]</td></tr>
<tr><td>Phase 3 (D22-30)</td><td>2.3[3]</td><td>2.6[3]</td><td>3.95</td><td>Not available</td><td>Not available</td><td rowspan="2">3.5</td></tr>
<tr><td>Phase 4 (D31-38)</td><td>2.6 (± 0.13)</td><td>3.1 (± 0.07)</td><td>3.19</td><td>Not available</td><td>Not available</td></tr>
</table>

[1]) Determined using a linear-plateau response model

[2]) Depending on the dietary Ca-content and on the use of microbial phytase

[3]) No effect on performance. Therefore lowest tested value was used

Table 22

Overview of recommended dietary absorbable phosphorus levels (g/kg) for minimal FCR of broilers per phase.

<table>
<tr><th>Source

Basis
Period</th><th>Current study

Calculated aP</th><th>Current study

Analysed aP</th><th>(Mello et al., 2012)

Calculated aP</th><th>(Runho et al., 2001)

Calculated aP</th><th>(Letourneau-Montminy et al., 2010)
nPP</th><th>(Venalainen et al., 2006)

Calculated aP</th></tr>
<tr><td>Phase 1 (D0-10)</td><td>5.4 (± 0.70)</td><td>4.1 (± 0.11)</td><td>4.8</td><td rowspan="2">4.5</td><td rowspan="2">3.3-4.1[1]</td><td rowspan="2">4.0[2]</td></tr>
<tr><td>Phase 2 (D11-21)</td><td>Not Available</td><td>4.4 (± 0.06)</td><td>3.6</td></tr>
<tr><td>Phase 3 (D22-30)</td><td>2.3[2]</td><td>2.6[2]</td><td>Not available</td><td>Not available</td><td>Not available</td><td rowspan="2">3.5[2]</td></tr>
<tr><td>Phase 4 (D31-38)</td><td>2.5</td><td>3.1</td><td>Not available</td><td>Not available</td><td>Not available</td></tr>
</table>

[1]) Depending on the dietary Ca-content and on the use of microbial phytase

[2]) No effect on performance. Therefore lowest tested value was used

Differences in requirements between studies can be partly explained by differences in the methods of calculating dietary aP contents. The values used in the current study were based on tabulated CVB values, which are obtained by determining retainable P contents of feed ingredients and recalculated to absorbable P contents. Mello et al. (2012) used for P-absorbability of feed ingredients a fixed value of 33%. This value is far below the P-absorbability values used in the current study, which ranged among diets from about 47 to 60%. An underestimation of the dietary aP content might probably also result in an underestimation of the aP requirement. Létourneau-Montminy et al. (2010) used nPP as a measure for aP-requirements, which is different from the current study based on absorbable P. As shown in Table 19, broilers are able to degrade phytate to a certain extent, and therefore nPP is underestimating the P-absorption of the diet. The by Létourneau-Montminy et al. (2010) provided requirements, therefore, also seem to underestimate the aP requirements of broilers. The used aP values of feed ingredients in the study of Venäläinen et al. (2006) were comparable with the CVB values, e.g. for wheat (35 vs. 39%), barley (47 vs 37%), soybean meal (42 vs 42%) and monocalciumphosphate (80 vs 85%). A possible reason for absents in clear relationships between dietary aP content and performance level of birds is the relative small test range in this study (100 to 137.5% of CVB recommendations).

4.3 aP-requirements based on tibia traits

In phase 1 of the current study, tibia breaking strength and tibia ash content were not affected by dietary treatment. For 1-10d of age, Mello et al. (2012) estimated the aP-requirement for maximal tibia ash content on 4.8 g/kg (quadratic model) or 3.8 g/kg (linear response plateau).
In phase 2 of the current study, tibia ash content was maximal at an aP-level of 112.5% of CVB (2012) requirement, which corresponded with a calculated aP-value of 3.8 g/kg. Based on the broken-stick approach, the required aP-content for maximal tibia ash were 3.8 and 4.0 g/kg calculated and analysed dietary aP, respectively. These values are in line with the calculated aP-value recommended by Mello et al. (2012) based on a linear response plateau model (4.1 g/kg). As shown in Table 20, Létourneau-Montminy et al. (2010) estimated a nPP-content in the range of 3.9 to 5.2 g/kg for maximal tibia ash contents, depending on dietary Ca-content and phytase supplementation. Létourneau-Montminy et al. (2010) estimated higher nPP contents for maximal tibia ash contents as compared to nPP contents for maximal BWG and FCR, which was not the case in the current study. In the current study, the aP-requirements for realizing maximal tibia ash weight, calculated as tibia weight x tibia ash content, were in phase 2 (4.95 g/kg), 3 (3.80 g/kg) and 4 (3.92 g/kg) higher than for maximal BWG or minimal FCR. In the study of Venäläinen et al. (2006) tibia ash content was highest in birds that were fed a starter diet with 4.5 g/kg aP.

In phase 3 of the current study, tibia ash content was maximal at an aP-level of 125% of CVB (2012) requirement, which corresponded with a calculated aP-value of 3.8 g/kg. Based on the broken-stick approach, the required aP-content for maximal tibia ash were 3.8 and 3.5 g/kg in dependence of the calculated and analysed dietary aP-contents, respectively. These values are in line with the by Mello et al. (2012) reported aP-value of 3.95 g/kg by use of a linear response plateau model, but considerably lower than the aP-value of 4.88 g/kg by use of a quadratic model.
In phase 4 of the current study, tibia ash content was maximal at an aP-level of 125% of CVB (2012) requirement, which corresponded with a calculated aP-value of 3.5 g/kg. Based on the broken-stick approach, the required aP-content for maximal tibia ash were 2.6 and 3.2 g/kg in dependence of the calculated and analysed dietary aP-contents, respectively. These values are in line with the by Mello et al. (2012) reported aP-value of 3.19 g/kg by use of a linear response plateau model, but considerably lower than the aP-value of 4.21 g/kg by use of a quadratic model. Based on tibia ash weight response to different dietary nPP levels, Dhandu and Angel (2003) estimated the nPP-requirement for broilers from 32 to 42 d of age on 2.0 (± 0.1) g/kg, and for broilers from 42 to 49 d of age on 1.6 (± 0.2) g/kg. In the study of Venäläinen et al. (2006) tibia ash content was highest in birds that were fed a finisher diet with 4.0 g/kg aP.
An overview of recommended dietary aP levels for maximal tibia ash, based on the current study and on available literature, is shown in Table 23.

Table 23
Overview of recommended dietary absorbable phosphorus levels (g/kg) for maximal tibia ash contents of broilers per phase.

Source	Current study	Current study	(Mello et al., 2012)	(Runho et al., 2001)	(Letourneau-Montminy et al., 2010)	(Venalainen et al., 2006)	(Dhandu and Angel, 2003)
Basis Period	Calculated aP	Analysed aP	Calculated aP	Calculated aP	nPP	Calculated aP	nPP
Phase 1 (D0-10)	3.0[1]	3.8[1]	3.8[2]				Not Available
Phase 2 (D11-21)	3.8 (± 0.03)	4.0 (± 0.03)	4.1-5.3[2]	4.5	3.9-5.2[3]	4.5	Not Available
Phase 3 (D22-30)	3.8 (± 0.03)	3.5 (± 0.15)	4.0-4.9	Not available	Not available	4.0	Not available
Phase 4 (D31-38)	2.6 (± 0.22)	3.2 (± 0.16)	3.2-4.2	Not available	Not available		2.0 (±0.1)[4]

[1]) No effect on performance. Therefore lowest tested value was used

[2]) Determined using a linear-plateau response model

[3]) Depending on the dietary Ca-content and on the use of microbial phytase

[4]) 1.6 (± 0.2) g/kg for broilers between 42 and 49 d of age

4.4 Carcass P content

The carcass P content of the birds in the current experiment, averaged over all treatments, as compared to the contents reported by several other authors, are presented in Figure 8.

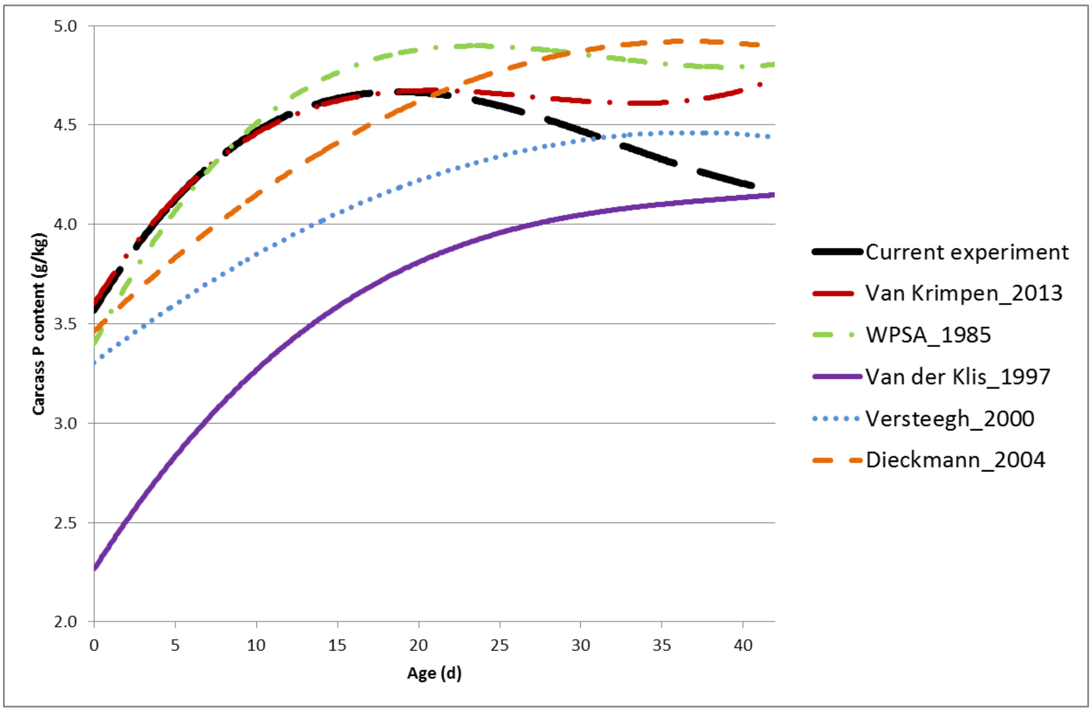

Figure 8 *Carcass P content of the birds in the current experiment, as compared to the contents reported by several other authors*

Up to 21 d of age, the carcass P contents in the birds of the current experiment are similar to our previous experiment (Van Krimpen et al., 2013). At d30 and d38, however, carcass P contents were reduced compared to our previous experiment. This is unexpected and not in line with our previous findings, or with values published by other authors. A clear explanation for this phenomenon was not found. Probably, birds deposited more fat at the end of the growing period, thereby diluting the carcass P content. Fat contents, however, are not determined in the sampes. Dieckmann (2004) reported an average P content of broilers of 4.8 g/kg, with higher values for male birds compared to females (5.0 vs. 4.6 g/kg), while comparable values were reported by WPSA (1985), ranging from 4.9 g/kg at 3 wk of age to 4.7 g/kg at 8 wk of age. In contrast, lower P contents at slaughter were reported by Van der Klis and Blok (1997) (4.0 g/kg) and Versteegh and Jongbloed (2000) (4.4 g/kg).

5 Conclusions

Table 24 summarizes per phase the current CVB (2012) aP requirements, the calculated aP levels for maximal BWG, minimal FCR, maximal tibia ash content. Requirements are based on calculated aP, which allows a comparison with tabulated aP-values for feed ingredients (CVB, 2011). Interpretation of analysed aP levels is difficult, because these values are not constant, but differed in dependence of the level of aP-supply of the birds. and the recommended aP level based on the results of the current study.

Table 24
Overview per phase of the current CVB (2012) aP requirements, the calculated aP levels for maximal BWG, minimal FCR, and maximal tibia ash content according to the broken stick model.

Source Period	CVB (2012)	Current study Maximal BWG	Current study Minimal FCR	Current study Maximal tibia ash
Phase 1 (D0-10)	4.0	5.4	5.4	3.0
Phase 2 (D11-21)	3.4	4.3	---	3.8
Phase 3 (D22-30)	3.0	---	---	3.8
Phase 4 (D31-38)	2.8	2.6	2.5	2.6

The conclusions are as follows:

- In phase 1, 2 and 4, growth performance responded linear to incremental dietary aP contents.
- In phase 1 and 2, FCR responded linear to incremental dietary aP contents, whereas FCR in phase 4 responded quadratic.
- In phase 4, the absolute tibia breaking strength responded linear to incremental dietary aP contents.
- In phase 1 and phase 2, the calculated dietary aP-levels for maximal BWG and minimal FCR according to the broken stick model were higher than the calculated aP-level for maximal tibia ash content.
- In phase 3, performance of the broilers was not affected by dietary aP content. For maximal tibia ash content, an aP-level of 3.8 g/kg was required.
- In phase 4, the calculated dietary aP-levels for maximal broiler performance and tibia ash content were similar (2.6 g/kg).
- Broilers were able to increase dietary P absorption at aP-levels below 112.5% (d21 and d30) or 125% (d10 and d38) of the CVB (2012) recommendations. P absorption was increased by increasing phytate degradation.
- Tibia breaking strength, expressed per g tibia or per mm tibia section, was in most cases not affected by dietary aP-content. In 38 day-old broilers, the absolute tibia breaking strength, as well as the breaking strength expressed per mm tibia section, was lower in birds fed the 75% aP-diets compared to birds fed the other diets.
- Footpad lesions, feather quality, and litter quality were not affected by dietary aP-levels.
- Average carcass P content was 4.5, 4.8, 4.5, and 4.2 g/kg at d10, d21, d30, and d38, respectively. At d10 and d21, carcass P content tended to increase with increasing dietary aP-content.
- In birds dissected at d10 and d21, carcass Ca content increased with increasing dietary aP-content. At d30 and d38, carcass Ca content was not affected by dietary treatments; average carcass Ca content was 6.0 and 5.6 g/kg, respectively.

This study showed that for realizing maximal performance, in phase 1 (d1-d10) 5.4 g calculated aP per kg of feed was required, in phase 2 (d11-d21) 4.3 g calculated aP per kg of feed, and in phase 4 (d31-d38) 2.6 g calculated aP per kg of feed. In phase 3 (d22-d30), performance of the broilers was not affected by the calculated aP content. For realizing maximal tibia ash content in phase 3, a calculated aP-level of 3.8 g/kg was required. In phase 1 and 2 of the current study, the dietary aP level required for maximal performance phase are about 30% higher compared to the current CVB recommendations. For these phases, aP recommendations should be reconsidered.

Literature

Applegate, T. J., and R. Angel. 2014. Nutrient requirements of poultry publication: History and need for an update. J. Appl. Poult. Res. 23(3):567-575.

Collins, K. E., B. H. Kiepper, C. W. Ritz , B. L. McLendon, and J. L. Wilson. 2014. Growth, livability, feed consumption, and carcass composition of the athens canadian random bred 1955 meat-type chicken versus the 2012 high-yielding cobb 500 broiler. Poult. Sci. 93:2953-2962.

CVB. 2011. Veevoedertabel 2011, centraal veevoederbureau. Lelystad, The Netherlands.

CVB. 2012. Tabellenboek veevoeding 2012. CVB-reeks nr. 50, Productschap Diervoeder, Den Haag, Augustus 2012.

Dhandu, A. S., and R. Angel. 2003. Broiler nonphytin phosphorus requirement in the finisher and withdrawal phases of a commercial four-phase feeding system. Poult. Sci. 82(8):1257-1265.

Dieckmann, A. 2004. Beiträge zur optimierung der phosphorversorgung von wachsenden broilern. ed.

Eeckhout, W., and M. Depaepe. 1994. Total phosphorus, phytate-phosphorus and phytase activity in plant feedstuffs. Anim. Feed Sci. Technol. 47(1-2):19-29.

Havenstein, G. B., P. R. Ferket, and M. A. Qureshi. 2003. Growth, livability, and feed conversion of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. Poult. Sci. 82(10):1500-1508.

ISO 5983. 1997. Animal feeding stuffs. Determination of nitrogen content and calculation of crude protein content. Kjeldahl method. Iso 5983. Int. Organ. Standardization, geneva, switzerland.

ISO 5984. 2002. Animal feeding stuffs. Determination of crude ash. Iso 5984. Int. Organ. Standardization, geneva, switzerland.

ISO 6496. 1998. Animal feeding stuffs. Determination of moisture and other volatile matter content. Iso 6496. Int. Organ. Standardization, geneva, switzerland.

ISO 11885. 1998. Water quality. Determination of 33 elements by inductively coupled plasma atomic emission spectroscopy. Iso 11885. Int. Organ. Standardization, geneva, switzerland.

Kestin, S. C., T. G. Knowles, A. E. Tinch, and N. G. Gregory. 1992. Prevalence of leg weakness in broiler-chickens and its relationship with genotype. Vet. Rec. 131(9):190-194.

Letourneau-Montminy, M. P., A. Narcy, P. Lescoat, J. F. Bernier, M. Magnin, C. Pomar, Y. Nys, D. Sauvant, and C. Jondreville. 2010. Meta-analysis of phosphorus utilisation by broilers receiving corn-soyabean meal diets: Influence of dietary calcium and microbial phytase. Animal 4(11):1844-1853.

Li, W., R. Angel, S. W. Kim, E. Jimenez-Moreno, M. Proszkowiec-Weglarz, and P. W. Plumstead. 2015. Impact of response criteria (tibia ash weight vs. Percent) on phytase relative non phytate phosphorus equivalance. Poult. Sci. 94(9):2228-2234.

Marounek, M., I. Bubancova, M. Podsednicek, D. Lukesova, and M. A. Ramadan. 2011. Activity of phytate dephosphorylation in cereals, legumes and oilseeds determined by colourimetric and isotachophoretic method. J. Anim. Feed Sci. 20(3):472-479.

Mello, H. H. D., P. C. Gomes, H. S. Rostagno, L. F. T. Albino, T. C. da Rocha, R. L. de Almeida, and A. A. Calderano. 2012. Dietary requirements of available phosphorus in growing broiler chickens at a constant calcium: Available phosphorus ratio. Rev. Bras. Zootecn. 41(11):2323-2328.

Myers, W. D., P. A. Ludden, V. Nayigihugu, and B. W. Hess. 2004. Technical note: A procedure for the preparation and quantitative analysis of samples for titanium dioxide. J. Anim. Sci. 82(1):179-183.

NRC. 1994. Nutrient requirements of poultry.

Runho, R. C., P. C. Gomes, H. S. Rostagno, L. F. T. Albino, P. S. Lopes, and P. C. Pozza. 2001. Available phosphorus requirement of male and female broilers from 1 to 21 days of age. Rev. Bras. Zootecn. 30(1):187-196.

Sauvant, D., J. M. Perez, and G. Tran. 2004. Tables de composition et de valeur nutritive des matie` res premie` res destine´ es aux animaux d'e´ levage: Porcs, volailles, bovins, ovins, caprins, lapins, chevaux, poissons. INRA Editions, Versailles, France,.

Short, F. J., P. Gorton, J. Wiseman, and K. N. Boorman. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim. Feed Sci. Technol. 59(4):215-221.

Singh, P. K. 2008. Significance of phytic acid and supplemental phytase in chicken nutrition: A review. Worlds Poult. Sci. J. 64(4):553-580.

Van der Klis, J. D., and M. C. Blok. 1997. Definitief systeem opneembaar fosfor pluimvee. L. CVB Documentatierapport nr. 20. Centraal Veevoederbureau, September 1997 ed.

Van Krimpen, M. M., J. T. M. Van Diepen, P. G. Van Wikselaar, P. Bikker, and A. W. Jongbloed. 2013. Effects of available phosphorus (ap), calcium/ap ratio, and growth rate on p deposition, p digestibility,

performance and leg quality in broilers. R. Wageningen UR Livestock Research, Wageningen, The Netherlands, 29 pp ed.
Venalainen, E., J. Valaja, and T. Jalava. 2006. Effects of dietary metabolisable energy, calcium and phosphorus on bone mineralisation, leg weakness and performance of broiler chickens. Brit. Poult. Sci. 47(3):301-310.
Versteegh, H. A. J., and A. W. Jongbloed. 2000. Het gehalte aan droge stof, as, stikstof, calcium, fosfor, kalium, koper en zink in vleeskuikens op 3 leeftijden. ed., Rapport ID-Lelystad no. 99.042, Lelystad.
Welfare Quality. 2009. Welfare quality; assessment protocol for poultry. L. ASG Veehouderij BV, The Netherlands ed.
WPSA. 1985. Mineral requirements for poultry - mineral requirements and recommendations for growing birds. (working group no. 2 - nutrition - of the european federation of the wpsa). Worlds Poult. Sci. J. 41(3):252-258.

Bijlage 1 Appendix 1 Diet compositions

Item		Phase 1		Phase 2	
		Low P	High P	Low P	High P
Ingredients (g/kg)					
Corn		295.0	295.0	248.5	248.5
Wheat		235.7	235.7	360.1	360.1
Rapeseed expeller		30.0	30.0	36.0	36.0
Soybean meal 50		331.5	331.5	255.4	255.4
Soybean oil		25.0	25.0	27.0	27.0
Poultry fat		22.0	22.0	20.0	20.0
Chalk		10.2	18.9	8.9	16.3
Sodium bicarbonate		3.3	3.3	2.9	2.9
Salt		1.3	1.3	1.0	1.0
Belfeed		0.1	0.1	0.1	0.1
DL-Methionine		2.3	2.3	2.0	2.0
L-Threonine		1.4	1.4	1.0	1.0
Maxiban		5.0	5.0	5.0	5.0
Diamol		21.8	-	18.3	-
L-Lysine		1.7	1.7	1.9	1.9
Broiler premix		5.0	5.0	5.0	5.0
Vit hy-D PX		0.4	0.4	0.2	0.2
Monocalcium phosphate		8.3	21.4	6.7	17.6
Calculated nutrients					
CP	g	220	220	195	195
Crude fat	g	72	72	71	71
Crude fibre	g	28	28	28	28
Ash	g	75	76	65	66
DM	g	886	887	883	884
Starch	g	335	335	378	378
Ca	g	6.6	12.1	5.7	10.3
P	g	5.6	8.5	5.0	7.5
aP	g	3.0	5.5	2.6	4.7
Na	g	1.5	1.5	1.3	1.3
K	g	9.8	9.8	8.6	8.6
Cl	g	1.7	1.7	1.6	1.6
Vit. A	ie	14000	14000	14000	14000
Vit. D3	ie	2900	2900	2900	2900
Vit. E	ie	60	60	60	60
Vit. D3 OH	Ie	2100	2100	1100	1100
Phytase	FTU	-	-	-	-
C18:2	g	27.2	27.2	27.3	27.3
Vit. K3	mg	1.5	1.5	1.5	1.5
Narasin	mg	50	50	50	50
Nicarbazin	mg	50	50	50	50
MEbr	MJ	12.18	12.18	12.40	12.40
dig. LYS	g	11.5	11.5	10.0	10.0
dig. MET	g	4.5	4.5	4.6	4.6
dig. M+C	g	7.1	7.1	7.3	7.3
dig. THR	g	7.0	7.0	6.8	6.8
dig. TRP	g	2.0	2.0	2.0	2.0
dig. ILE	g	7.0	7.0	6.9	6.9

Item		Phase 3		Phase 4	
		Low P	High P	Low P	High P
Ingredients (g/kg)					
Corn		215.1	215.1	190.0	190.0
Wheat		390.0	390.0	424.0	424.0
Rapeseed expeller		48.0	48.0	64.0	64.0
Soybean meal 50		247.4	247.4	228.0	228.0
Soybean oil		17.7	17.7	17.0	17.0
Poultry fat		36.0	36.0	40.0	40.0
Chalk		7.7	14.0	7.1	13.4
Sodium bicarbonate		2.5	2.5	2.4	2.4
Salt		1.3	1.3	1.4	1.4
Belfeed		0.1	0.1	0.1	0.1
DL-Methionine		1.3	1.3	1.2	1.2
Salinomycine		5.8	5.8	-	-
Diamol		15.8	-	15.8	-
L-Lysine		1.2	1.2	1.1	1.1
Broiler premix		5.0	5.0	4.0	4.0
Vit hy-D PX		0.1	0.1	-	-
Monocalcium phosphate		5.0	14.4	3.9	13.3
Calculated nutrients					
CP	g	194	194	191	191
Crude fat	g	78	78	82	82
Crude fibre	g	30	30	31	31
Ash	g	60	61	58	58
DM	g	883	884	883	884
Starch	g	375	375	376	376
Ca	g	5.1	9.0	4.6	8.6
P	g	4.7	6.8	4.5	6.6
aP	g	2.3	4.1	2.1	3.9
Na	g	1.3	1.3	1.3	1.3
K	g	8.5	8.5	8.3	8.3
Cl	g	1.6	1.6	1.6	1.6
Vit. A	ie	12000	12000	9600	9600
Vit. D3	ie	2400	2400	1920	1920
Vit. E	ie	50	50	40	40
Vit. D3 OH	Ie	600	600	-	-
Phytase	FTU	-	-	-	-
C18:2	g	25.4	25.4	25.7	25.7
Vit. K3	mg	1.5	1.5	1.2	1.2
Salinomycine	mg	69.6	69.6	-	-
MEbr	MJ	12.60	12.60	12.79	12.79
dig. LYS	g	9.4	9.4	9.1	9.1
dig. MET	g	4.1	4.1	4.2	4.2
dig. M+C	g	7.1	7.1	7.2	7.2
dig. THR	g	6.2	6.2	6.3	6.3
dig. TRP	g	2.2	2.2	2.2	2.2
dig. ILE	g	7.4	7.4	7.4	7.4

To explore the potential of nature to improve the quality of life

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Together with our clients, we integrate scientific know-how and practical experience to develop livestock concepts for the 21st century. With our expertise on innovative livestock systems, nutrition, welfare, genetics and environmental impact of livestock farming and our state-of-the art research facilities, such as Dairy Campus and Swine Innovation Centre Sterksel, we support our customers to find solutions for current and future challenges.

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