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EVALUATION OF THE AVAILABILITY OF DIFFERENT MINERAL PHOSPHORUS SOURCES IN BROILERS

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LIST OF ABBREVIATIONS

AD SoS	Amplitude-dependent speed of sound
ANOVA	Analysis of variance
aP	Available phosphorus
AP	Alkaline phosphatase
ATP	Adenosintriphosphat
BBS	Bone breaking strength
BD	Basal diet
BMC	Bone mineral content
BMD	Bone mineral density
BW	Body weight
BWG	Body weight gain
CA	Crude ash
Ca^{2+}	Blood ionized calcium
CC	Cortical content
CcA	Citric acid
CD	Cortical mineral density
CP	Crude protein
CT	Calcitriol
DCP_a	Anhydrous dibasic calcium phosphate
DEXA	Dual-energy X-ray absorptiometry
DFP	Defluorinated phosphate
DM	Dry matter
DMCP	Dimonocalcium phosphate
EU	European Union
FA	Foot ash
FTU	Phytase units
GIT	Gastrointestinal tract
HPIC	High-performance ion chromatography
i.e.	That is
IP_6	Inositol-6-phosphate
MCP	Monobasic calcium phosphate
MDCP	Monodicalcium phosphate

ME	Metabolizable energy
MSP_a	Anhydrous monosodium phosphate
n	Number of observations
N	Newton
NAC	Neutral ammonium citrate
nPP	Non-phytin phosphorus
P	Phosphorus
pc	Prececal
P_i	Blood inorganic phosphate
PP	Phytin phosphorus
PTH	Parathyroid hormone
QCT	Quantitative computed tomography
r	Correlation coefficient
r^2	Goodness of fit
RBA	Relative biological availability
RBV	Relative biological value
SAS	Statistical Analysis System
SBM	Soybean meal
SD	Standard deviation
SEM	Standard error of the means
SSI	Polar Strain Strength Index
TBA	Tibia ash
TCT	Thyrocalcitonin
TD	Total mineral density
TMTA	Tarsometatarsal ash
TOA	Toe ash
TP	Tibia phosphorus
UV	Ultraviolet
WBP	Whole body phosphorus
wk	Weeks

CHAPTER 1

GENERAL INTRODUCTION

1. GENERAL INTRODUCTION

About 95% of the phosphate rock mined is used to produce fertilizers, animal feeds, and pesticides, which indicates that the development of agriculture is the driving force for phosphate rock mining (Cisse and Mrabet, 2004). Phosphorus (**P**) in animal feed originates from feed materials including inorganic feed phosphates (IFP, 2006). According to the IFP (2009), the annual total use of inorganic feed phosphates is estimated to reach 1.4 million tons for the EU-25, or 0.25 million tons expressed as total P. This is almost 11% of the total P consumed by livestock via feed materials. However, phosphate may accumulate in the soil and leach if the concentration becomes too high, with negative consequences for surface waters, such as lake eutrophication (Rodehutscord, 2009). With respect to the needs of the animals and the environmental load, poultry production systems in many countries aim to optimize the use of dietary P (Rodehutscord and Dieckmann, 2005). In general, poultry contribute around 16% to the total P in livestock manure in the EU (IFP, 2009). Production of poultry meat has increased since 1991 by an average of 232 thousand tons per year in the EU-15 (IPPC, 2003). Broiler meat accounts for about 85% of total poultry meat worldwide (Huyghebaert et al., 2009). By far, the majority of poultry meat farms in the EU are also part of the production chain for chicken broilers (IPPC, 2003). Thus, broilers are the biggest consumer of inorganic feed phosphates in the poultry sector.

P is one of the most opulent minerals in a bird's body and, next to Ca, the basic component for the formation of bone tissue. Its orthophosphate form (PO_4^{3-}) plays a key role in numerous metabolic reactions. P is needed for normal muscle growth and egg formation; it is a component of nucleic acids and of phospholipids; it is a component or activator of enzyme systems, and aids in maintaining osmotic and acid-base balance; it also is a factor in energy metabolism (ATP), amino acid metabolism, and protein synthesis (Coon et al., 2002). A marginal P supply with the feed for chicks may have a negative effect on performance, health, and bone development. Gillis et al. (1948) were the first authors who described the effects of severe P deficiency in young chicks. Having fed a purified basal diet (0.03% P) without additional P from day one post hatch, all the chicks died within 5-12 days. During the last stages of the P deficiency, the chicks lied on their sides and were not able to stand up. The authors concluded that young chicks require an immediate source of available P for the maintenance of vital functions. The results were the same whether or not Ca was added to the basal diet.

Plant feed ingredients are the basis for broiler diets. However, P in plant ingredients is largely present in the form of phytic acid and its salts (Eeckhout and de Paepe, 1994), and in this form only partly available to poultry. Thus, a supplementation of inorganic P to the plant-based diets is necessary for meeting a bird's requirement for this element. The more precise the supply of dietary P has to be adjusted to the specific requirement of available P, the better the knowledge about the availability of P from feed ingredients has to be (Rodehutscord, 2009). Therefore, the knowledge of P availability from various inorganic phosphate sources is a basis for the usage of optimal P levels in poultry feed.

P is a very expensive mineral in poultry diets (Singh, 2008). Supplemental phosphates overall obtain circa 60% of the non-phytin P needs of the chick (Waldroup, 1999). No standard evaluation method exists in the EU to differentiate between the digestibility[1] of inorganic feed phosphates (IFP, 2009). An adequate P supply without P excess remains one of the most important issues of broiler nutrition. In order to gain clarity and to enable the comparability of data, definitions and a system of available P which includes requirement data and raw material is needed (Rodehutscord, 2009). Thus, choosing an appropriate response criterion to measure the availability of feed P could be considered as the first step in this long process. Due to the relatively high cost of adding P to diets, in contrast to Ca, there has been a large amount of research dedicated to determining the necessary dietary levels and quality of P sources in poultry diets (Coon et al., 2002). However, scientists have used different methods and criteria in the evaluation of P availability. Thus, the data obtained is mostly impossible to compare. For this reason, a standard protocol for animal studies on P availability and phytase efficacy must be developed (Rodehutscord, 2009).

Other factors such as the type of diet used in experiments may also play a significant role in the determination of P availability, and clarifying of this issue is a cornerstone in establishing a standardized system of P evaluation in poultry. It is still not clear what impact a supplemental inorganic phosphate may have on the phytin P hydrolysis or, conversely, on the digestive tract of broilers, and how it may affect investigated results.

This thesis aims to help clarify some crucial questions in the evaluation of mineral P sources for broilers.

[1]In the report (IFP, 2009), digestible P = the difference between intake of P and excretion of P via the faeces and the urine in case of poultry.

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CHAPTER 2

LITERATURE REVIEW

2. LITERATURE REVIEW

2.1. INTRODUCTION

In the study of mineral metabolism, it is generally recognized that the total content of a mineral element in a particular feed ingredient or complete diet has little significance unless it is qualified by a factor indicating the biological availability of the element to animals (Peeler, 1972). The need for inorganic P supplements has led to the development and evaluation of various P supplements (Sullivan and Douglas, 1990). Various response criteria have been used in the estimation of P availability. Early experiences with inorganic supplements and subsequent studies clearly indicated that feeding tests or bioassays were necessary (Sullivan and Douglas, 1990).

Moreover, there has been a lot of confusion in the terminology used in P research in poultry for the last 70 years. Nowadays, for instance, the definition of available P (**aP**) differs between various evaluation systems (NRC, 1994; GfE, 1999; CVB, 1997; INRA, 2004), as well as between different researchers. Additionally, each of these systems has used different approaches for investigating P availability. In order to avoid any further confusion, the following definitions[2] are suggested here and will be used in this thesis:

- aP is that part of dietary total P that, at a marginal level of P supply, can be utilized to cover the P requirement of the animal. Availability describes a potential of a diet or a raw material (Rodehutscord, 2009).
- Total P comprises all P contained in a feed as chemically analyzed, irrespective of the binding form.
- Phytin P (**PP**) is all the P contained as phytic acid (inositol hexakisphosphate, **IP_6**) and its salts (phytate).
- Non-phytin P (**nPP**) is the difference between analyzed total P and analyzed PP.
- Retainable P is that proportion of the dietary total P that is deposited in the body of an animal.
- Precaecally digestible P is that proportion of the dietary total P that is not recovered in the content of the terminal ileum. It is determined by including an indigestible marker in the feed.

[2] Originate from a group of experts who work on a standard trial protocol for determination of P availability in poultry (Working Group 2 – Nutrition – of the European Federation of branches of the World's Poultry Science Assiciation (Sub-Committee on the Mineral Requirements of poultry), 2012).

- Relative bioavailability (**RBA**) of P uses responses in bone data (ash content, P content, breaking strength, etc.) or other biological data (e.g. body weight gain, blood inorganic P concentration). Responses to a certain P source are compared with the response to a standard reference P source.

The objective of this review is to give an overall view of the various methods used for the estimation of P availability.

Methods for the estimation of P availability can be divided as follows:

1) Qualitative measurements of P availability (bone, blood, and performance studies);
2) Quantitative measurements of P availability (retention, pc digestibility, and comparative whole body analysis studies);
3) *In vitro* tests (solubility studies).

2.2. EVALUATION METHODS IN POULTRY

2.2.1. QUALITATIVE MEASUREMENTS OF P AVAILABILITY

According to Nelson and Peeler (1961), in order to obtain valid comparisons of phosphates, it is necessary that (1) a sensitive criterion of measure is used, (2) a critically P-deficient diet is fed, (3) the levels of added P are not in excess of the animal's requirement, and (4) a suitable standard is used for comparison with the phosphates tested.

2.2.1.1. BONE CRITERIA

Bone development has been considered for years as one of the most critical tests for estimating bioavailability of Ca and P (Ammerman, 1995). The bone criteria are important criteria for estimating P availability because about 80% of P is located in the skeleton and the P content of bones remains almost constant (De Groote and Huyghebaert, 1997). The zone of proliferation in the developing bone of the young chick is especially sensitive to nutritional deficiencies and is quickly influenced by a P deficiency (Nelson and Walker, 1964). However, the outcome of the experiments based on bone data depends upon the quality of the reference P source and provides only relative values of P availability (Rodehutscord, 2009). In usual assays for testing phosphate supplements, the test sources are added to a P-deficient diet to supply graded sub-optimum levels of P (Harms et al., 1967). The standard phosphate used is assigned a biological value of 100. The relative biological values of the test phosphates are

determined by a comparison of the results obtained for the respective bone response criterion among all P sources used and are expressed in relation to the biological value of the standard P source (Leske and Coon, 2002). However, results have varied quite widely between different researchers due to differences in protocol, the reference phosphate, and the origin of the test phosphates used (IFP, 2006).

Bone ash and P

Most projects in the last 70 years focused on bone ash. The length of the experimental period differed largely between the studies (Table 1).

Table 1: The length of the experimental period in assaying supplements as a source of P based on bone ash in poultry

Criterion	Experimental period, d	Reference
Tibia ash	10[1]	Ammerman et al. (1960)
	11	Coon et al. (2007)
	14	Pensack and Stokstad (1961), Waibel et al. (1984), Akpe et al. (1987), Onyango et al. (2003)
	16	Hurwitz (1964)
	20	Pensack (1974), Wozniak et al. (1977)
	21	Nelson and Walker (1964), Jensen and Edwards (1980), Huyghebaert et al. (1980), Nelson et al. (1990), Leske and Coon (2002)
	28	Gillis et al. (1948, 1954), Summers et al. (1959), Vandepopuliere et al. (1961) Nelson and Peeler (1961), Waldroop et al. (1965), Rowland et al. (1967), Struwe et al. (1976)
	35	Miller and Joukovsky (1953), Motzok et al. (1956)
Femur ash	26	Hemme et al. (2005)
	28	Gardiner (1962)
Foot ash	7	Garcia et al. (2006)
	14	Garcia and Dale (2006)
Tibia ash, toe ash	10	Yoshida and Hoshii (1977), Yoshida and Hoshii (1983)
Tibia ash, toe ash	21	Ravindran et al. (1995), Kornegay et al. (1996), Kornegay and Yi (1999)
Toe ash	21	Fritz et al. (1969), Soares et al. (1978), Potchanakorn and Potter (1987), Potter (1988)
Toe ash	28	Johnson et al. (1953)
Tibia ash, toe ash, foot ash	17	Mendez et al. (1998)
Tibia ash, toe ash, foot ash	21	Yan et al. (2005)

[1] A 4-day P depletion period followed by a 6-day P supplemental period

However, bone ash responses may be affected by the duration of the experiment. Ammerman et al. (1961) used a degerminated corn-soybean meal-diet in 10 and 28 d bioassays for evaluating P supplements and reported that 10 d bioassay was less sensitive than a 28 d bioassay for generating data for a tibia ash response curve.

Following the outbreak of the Second World War, there was an extreme shortage of phosphate-carrying materials, and it was not possible to secure sufficient phosphate from defluorinated products or bone meal to use in poultry ratios (Miller and Joukovsky, 1953). Thus, the evaluation of inorganic P supplements in the 1940s as well in the 1950-1960s was mostly dedicated to the identification of products which could be applicable as feed phosphates for poultry. Bird and Caskey (1943) evaluated amorphous calcium metaphosphate as a P supplement for chicks. The researchers concluded that the P of calcium metaphosphate was utilized effectively based on tibia ash data. Matterson et al. (1945) compared the P availability (based on tibia ash) of raw rock phosphate and fused rock phosphate, and concluded that both phosphates were as available as tri-calcium phosphate. The so-called "replacement" technique was employed by some scientists in the 1950s for evaluating different P sources (Johnson et al., 1953; Motzok et al., 1956, 1957; Day and Hill, 1959; Watts and Miner, 1959; Baruah et al., 1960a; Baruah et al., 1960b). Thereby a part of a reference phosphate was replaced by a portion of a test source in the diet in order to investigate effects on different response criteria including tibia ash. Nelson and Peeler (1961) noticed that such biological assays did not give a critical measure of phosphate availability because the comparative availabilities of combinations of phosphates were not determined. In contrast, Gillis et al. (1954) were the first who employed tibia ash concentration as a reference criterion for quantifying the availability of P for chicks. The method was based on relating percentage of tibia ash from chicks fed a given test supplement to that of chicks fed a beta-tricalcium phosphate as a reference standard, to establish a relative biological value. Nelson and Walker (1964) reported in a summary of 82 experiments from literature that in the type of assays such as used by Gillis et al. (1954) an extremely steep response curve to beta-tricalcium phosphate was determined and a slope that was highly reproducible from assay to assay. Hurwitz (1964) developed a method which was based on the determination of the regression slope of tibia P as a function of total P intake. Field et al. (1974) found that Ca percentage in bone ash varies little among species or anatomical location of bones, which is also valid for P because of their relationship as the components of hydroxylapatite (Ca:P =

2:1) (Field, 1999). Ash concentration in bone may be decreased by low Ca or P diets, but the Ca concentration in ash still remains constant at approximately 37% (Field, 1999).

Not only tibia but also femur bone mineralization was used as a response criterion in the evaluation of dietary P or its adequacy in broilers (Gardiner, 1962; Orban and Roland, 1992; Moran and Todd, 1994; Chen and Moran, 1995; Hemme et al., 2005; Angel et al., 2006). Applegate and Lilburn (2002) demonstrated that the femur and tibia exhibited different patterns of development during a commercial growing period. Moran and Todd (1994) and Angel et al. (2006) showed in broilers that femur measurements were even more sensitive than those of the tibia.

Obtaining the tibia or femur ash is a quite laborious technique. Cambell et al. (1945) mentioned that among the disadvantages of tibia ash method were the dissection and cleaning of the bones and the lengthy extraction period. Baird and MacMillan (1942) proposed to use toe rather than tibia ash as a criterion of calcification in vitamin D determination in chicks. The use of toes made it possible to carry out the test without sacrificing the bird and to eliminate the labor and time involved in removing and cleaning the tibiae (Baird and MacMillan, 1942). Yoshida and Hoshii (1977) used toe ash for determining P availability in growing chicks. The relationship between dose, i.e., dietary P level and the toe ash concentration was linear in the range from 0 to 0.3% of added P. A simplified bioassay procedure of 10 d feeding period was recommended, which was a slope ratio assay of linear regression lines between added P and toe ash concentration. A high correlation between toe and tibia ash was also reported (Yoshida and Hoshii, 1983). Mendez and Dale (1998) and Mendez et al. (1998) proposed foot ash as a rapid means to quantify the degree of bone mineralization in broilers. Garcia and Dale (2006) confirmed that the dietary P levels affected bone mineralization, and that the degree of mineralization could reliably be reflected by foot ash. Foot ash was found to be as reliable as tibia bone ash in reflecting the degree of mineralization in chicks during the first 14 d of age. A high degree of relationship between tibia ash, toe ash, and foot ash was reported by Mendez et al. (1998) and Yan et al. (2005) (Table 2).

Table 2: Relationship (r^2) between tibia ash and toe or foot ash in broiler chicks

Criterion	Mendez et al. (1998)	Yan et al. (2005)	Garcia and Dale (2006)
Toe ash	0.82	0.88	-
Foot ash	0.85	0.92	0.92

Ammerman et al. (1961) used the ash content of the lower beak for the measurement of P availability in different inorganic phosphate sources. However, this criterion was not as sensitive as the ash content of the tibiae.

Ravindran et al. (1995) applied various response criteria in evaluating biological availability of P for broilers. The criteria estimated included body weight gain (**BWG**), toe ash, tibia ash, tibia-specific gravity, tibia shear force, toe shear force, and metatarsal shear force; weight, length, diameter, and volume of tibia; weight, volume, and specific gravity of metatarsus; as well as volume and specific gravity of toe. The authors concluded that the chosen response criterion would considerably influence the relative availability estimates of a P source.

Nelson et al. (1990) pointed out that there was no standard method for conducting a biological value assay for P; thus, the differences in procedures that might influence the biological value of an individual sample included experimental species, reference phosphate, criteria of response, and Ca level in the test diets.

Bone breaking strength

Rowland et al. (1967) were the first who used bone breaking strength (**BBS**) as an indicator of dietary P and Ca adequacy. A correlation coefficient of 0.98 was found when the average tibia ash was correlated with the average breaking strength and of 0.63 when calculations were made on an individual bird basis. The authors suggested that the BBS was a good measure for dietary Ca and P level, as was tibia ash. Since then, BBS has been widely employed as response criterion for the evaluation of dietary P in growing chicks (Hayes et al., 1979; Huyghebaert et al., 1980; Ketels and De Groote, 1988; Burnell et al., 1990; Chung and Baker, 1990; Orban and Roland, 1992; Coffey et al., 1994; Ravindran et al., 1995; Lima et al., 1997; Fernandes et al., 1999; Leske and Coon, 2002; Hemme et al., 2005; Coon et al., 2007). However, a poor sensitivity of bone breaking strength or shear force was also reported by Huyghebaert et al. (1980), Ravindran et al. (1995), and Kornegay and Yi (1999). Furthermore, values for BBS may be affected by the type of instrument used, procedures used to prepare the bones for testing, as well as by physical and mechanical properties of bones (Orban et al., 1993). The age of the birds and the P supply in the earlier phase of growth may be also of importance. Moreover, bone breaking strength can be a sensitive measure when applied to young, rapidly growing animals, varying linearly with the quality and availability of the supplementary P (IFP, 2006).

Bone densitometry methods

The search for precise, accurate, and rapid assays, which could be able to replace bone ash in mineral evaluation, continued. Meyer et al. (1968) adapted the Cameron-Sorenson technique for measuring the bone mineral content (**BMC**) in humans to chickens. BMC was determined by measuring the transmission of a monoenergetic photon beam through the right tibia with a scintillation detector. The radioactive source was ^{125}I, and the mineral content was expressed as bone mass unit. The correlation between bone mass units and ash weight per cm bone was 0.955. The bone mass determined on a thin cross section of the bone was representative of the total mineral content of the bone. Akpe et al. (1987) examined the precision of bone densitometry (single photon absorptiometry) and bone ash methodologies as response criteria in the measurement of the bioavailability of P from various supplements for turkeys. Coefficients of correlation and variation and *F*-ratios were used for evaluation. The authors reported the correlation coefficient for percentage bone ash and bone density to be 0.986. In addition, as indicated by the *F*-ratios for testing treatment effects, bone densitometry was better able to detect differences among P sources than bone ash. Relative biological availabilities of P from various supplements were about the same based on the two methods.

Dual-energy X-ray absorptiometry (**DEXA**) has been used as an invasive or noninvasive technique for measuring bone mineral status in poultry (Onyango et al., 2003; Hester et al., 2004). Onyango et al. (2003) determined the BMC and bone mineral density (**BMD**) of defrosted tibiae in 3-wk-old broilers fed low-, medium- and adequate-P diets. The authors found that bone densitometry criteria were highly correlated to bone ash (0.92 and 0.93 for BMC and BMD, respectively). They concluded that BMC and BMD might be used to predict percentage tibia ash in broilers. Hester et al. (2004) validated that densitometry can detect differences in BMD *in vivo*, as demonstrated in hens fed varying concentrations of dietary Ca. BMD obtained from densitometric scans of live birds correlated well with the BMD readings of excised bones and other bone measurement tests such as bone breaking force and bone ash.

DEXA also allows measuring the whole body mineral content and density (Mitchell et al., 1997; Angel et al., 2006). Mitchell et al. (1997) reported that the ratio of DEXA BMC to total body ash was 0.77; however, the correlation between the two was only 0.46.

By using the DEXA method, only areal determination of the bone's mineral density (g/cm^2) is possible. A quantitative computed tomography (**QCT**) allows measuring the volumetric bone density in the sense of mass per unit of volume (g/cm^3). So cortical and trabecular density values (g/cm^3) can be measured. Korver et al. (2004) concluded that the

QCT was appropriate to follow changes in BMD in growing meat-type birds as they grow and remodel their skeletal structures to support increased body mass.

Other techniques such as digitized fluoroscopy (Fleming et al., 2000; Fleming et al., 2004) and amplitude-dependent speed of sound (**AD SoS**) (Fleming et al., 2004) were used to assess skeletal integrity in hens. Fleming et al. (2004) measured AD SoS in the distal end of the first phalanx of the third toe in hens. The authors concluded that AD SoS reflected other bone measurements (peripheral QCT and shear strength measured postmortem) and rapidly detected poor bone quality.

2.2.1.2. BLOOD CRITERIA

Gardiner (1962) investigated the relationship between dietary P level and the level of plasma inorganic P (**P_i**) of chicks in four experiments using corn-wheat-soybean meal-based diets. He concluded that the level of plasma P_i responded linearly with dietary increases in supplemental P up to 0.3%. Hurwitz (1964) also arrived at similar results. Plasma P_i was determined to be a useful measure for the estimation of the relative P availability and correlated well with tibia P. More recently, other investigations used plasma P_i (Lima et al., 1997; Fernandes et al., 1999) and serum P_i (Rama Rao et al., 1999; Hemme et al., 2005) for the evaluation of dietary P. Manangi and Coon (2006) showed with colostomized broilers that increasing P intake produced increasing P levels in the plasma that eventually plateau with adequate P intake.

Summers et al. (1959) were the first who tried to estimate P availability based on responses obtained from plasma alkaline phosphatase (**AP**) activity in growing chicks. However, the plasma AP did not prove to be a satisfactory criterion. Hurwitz and Griminger (1961) studied the response of plasma AP to Ca intake in laying hens and growing chicks. The possible use of AP activity for the determination of Ca adequacy was proposed. Data reported by Boyd et al. (1983) indicated that AP was a sensitive index potentially useful in P availability studies for swine. Afterwards, a few studies were conducted using AP as response criterion in the evaluation of dietary P in broilers (Lima et al., 1997; Viveros et al., 2002). However, the levels of plasma P_i and Ca are regulated by the vitamin D-endocrine system (parathyroid hormone, thyrocalcitonin, and calcitriol) (Auman, 2003). Gueguen (1999) and De Groote and Lippens (2002) pointed out that AP and serum/plasma P were not suitable for determining P bioavailability in poultry.

2.2.1.3. PERFORMANCE CRITERIA

Growth response in young chicken has been used as the primary criterion for determining bioavailability of several macro and micro mineral elements (Ammerman, 1995). Mostly together with bone ash, performance has been used as response criterion in P evaluation since the 1940s. Body weight (**BW**) has been one of the relevant criteria in assessing the relative bioavailability of phosphate sources. Gillis et al. (1948) determined the relative availability of P in chicks from different phosphate sources using BW at 4 wk of age and mortality together with tibia ash. Motzok et al. (1956) reported improved feed efficiency (feed/gain) with increasing P supplementation. Vandepopuliere et al. (1961) reported that the growth rate was as good as the criterion bone ash when used for determining the P adequacy of diets. Broilers are often more sensitive in their performance to differences in mineral supply than other animals, because they have low body reserves of minerals and a high growth rate (Jongbloed and Kemme, 2002). Nevertheless, Nelson and Walker (1964) showed that growth was less sensitive than bone ash as a criterion for evaluating phosphates; it was even more inaccurate when the level of dietary P almost met the chick's requirement for this element.

2.2.1.4. COMBINED RESPONSE CRITERIA

There was an attempt to combine several response criteria in P evaluation. Sullivan (1966) reported a triple response method for determining the relative availability of P sources using young turkeys. This approach involved percentage tibia ash, BW, and feed efficiency. Soares et al. (1978) applied feed consumption, BWG, and toe ash to assess P availability. However, as noticed by Peeler (1972), the method of combining various criteria into one value greatly reduces the sensitivity of the assay. This is expected, because the different criteria of response provide different relative biological values of P from a given source.

2.2.2. QUANTITATIVE MEASUREMENTS OF P AVAILABILITY

2.2.2.1. RETENTION AND PRECECAL DIGESTIBILITY

Bio-assays, based on growth, bone, or blood criteria, are able to provide the relative values of P availability. Therefore, the data are more of a qualitative than of a quantitative nature (Peeler, 1972). Thus, the information obtained from a relative biological availability assay has limited value for a nutritionist formulating diets (Coon et al., 2002). The nutritionist needs an actual retention value for key minerals to assess the true impact of dietary formulations on animal performance and on the elements remaining in animal excreta (Coon et al., 2002). The retention of P may be measured either by the complete excreta collection or by calculating the

retention value with the help of an indigestible marker. Nikolaiczuk et al. (1949) reported P retention values from organic and inorganic sources determined in balance trials with broilers over the age interval of one to seven weeks. Edwards and Gillis (1959) used a chromic oxide indicator method for evaluating the P availability in Comb Leghorn cockerels fed a blood-fibrin-gelatin-starch diet. Reasonably good agreement was obtained between the results of this method and the tibia ash method. Nwokolo et al. (1976) applied purified mineral-free diets for estimating the true availability of Ca, P, and other minerals in soybean meal, cottonseed meal, rapeseed and palm kernel meal in tests with 4-wk-old broilers. Availability of the minerals was determined by the amount of the mineral retained (%) with an adjustment for endogenous fecal excretion. Sibbald (1982) described the theoretical basis for extending the true metabolizable energy methodology to the measurement of bioavailable minerals. Van der Klis and Versteegh (1996) developed a method for evaluating P availability in feedstuffs for poultry diets. The P availabilities in commonly used feedstuffs were measured in 3-wk-old male broilers under standardized conditions. The test feedstuff was the only dietary source of P present in the purified experimental diets which were standardized at 1.8 g aP and 5 g Ca per kg feed. After 10 d of feeding the experimental diets, a 3-d balance period followed. A similar technique was used by De Groote and Huyghebaert (1997) who investigated the retention of P from three different feed phosphates in two balance trials using a nearly P-free purified basal diet. Each balance trial was carried out according to the European Reference Method (Bourdillon et al., 1990) consisting of a 7-d period of adaptation to the respective experimental diets and a 4-d main balance period with restricted feeding and total excreta collection.

Leske and Coon (2002) developed a 5-d bioassay with an acid insoluble ash marker for retainable P determination. Broilers were acclimated to cages and test diets for 3 d prior to initiating a 48-h excreta collection period. Retainable P values of feed-grade calcium phosphates were reported. Rodehutscord and Dieckmann (2005) used a 9-d balance trial with quantitative excreta collection to investigate the P utilization from a monobasic calcium phosphate (**MCP**) in different poultry species. The balance trial consisted of a 4-d lasting period of adaptation to the respective experimental diet and a 5-d lasting main balance period with restricted feeding and complete excreta collection.

The digestibility until the end of the ileum (prececal (**pc**) digestibility) is an established method for measuring protein quality in poultry (Rodehutscord, 2009). It is preferred because values are unaffected by post-ileal microbial activity. It also implies that the contribution of the urine can be excluded (Rodehutscord, 2009). Hurwitz et al. (1978)

measured P pc digestibility in young turkeys at the lower half of the ileum with the aid of yttrium-91 as a reference substance. The phosphate level in the diets was varied by supplementation of monocalcium phosphate. Grimbergen et al. (1985) and Kornegay et al. (1996) in young turkeys, Ketels and De Groote (1988), and Dickmann (2004) in broilers assessed the pc digestibility of P in various inorganic feed phosphates. Thus, the pc digestibility can be developed as an alternative tool to measure P availability, with the advantage of being less sensitive to the P level of the diet (Rodehutscord, 2009). This can be of advantage in studies on P availability, because urinary P excretion is the major pathway if intake is above the requirement but is negligibly low below the P requirement (Rodehutscord, 2009). Moreover, the response in P pc digestibility to increments in dietary P concentration is linear in a wider range of dietary P than the response in P retention (Rodehutscord et al., 2012).

2.2.2.2. P CONTENT OF THE WHOLE BODY

The total amount of a mineral that is retained in the animal body is one of the best response criteria for those minerals with a low mineral turnover such as Ca and P if the animals are fed below their mineral requirement (Jongbloed and Kemme, 2002).

Dieckmann (2004) used a comparative whole body analysis to quantify P retention from monobasic calcium phosphate supplemented to a low P basal diet at different P levels. Hemme et al. (2005) determined the retention of Ca and P in broilers fed four different inorganic P sources by analysis of body composition. At the beginning of the trial, three pool samples were completely analyzed (whole-body analysis) to obtain basic information on the Ca and P contents in the whole body. At the end of the trial, chemical body composition (e.g. Ca and P content) was analyzed to obtain information on the retention of both minerals in the experimental period. However, this technique is very laborious.

2.2.3. *IN VITRO* TESTS

Looking for quicker and cheaper methods which could substitute expensive and long-lasting bioassays, it was attempted to test P availability *in vitro*. These tests concern inorganic phosphates and are not applicable to organic P of either plant or animal origin (Gueguen, 1999). Conflicting results have been reported regarding the suitability of such tests in estimating bioavailability of phosphates (Waldroup, 1999).

Gillis et al. (1948) compared solubility data for different phosphates in 0.4% HCl with the biological availability data of the same materials. The authors concluded that solubility

had a limited usefulness in estimating availability for animals. For instance, rather soluble *alpha*, *beta*, and *gamma* calcium pyrophosphates (68.8%, 54.2%, and 73.4% total soluble P, respectively) were completely biologically unavailable. There was no significant difference in the survival time of chicks receiving these supplements and those receiving the unsupplemented basal diet (0.03% P), even when a sufficient quantity of those compounds was used to raise the P content of the diet to 0.8%. Gillis et al. (1962) compared the solubility of various sources of calcium phosphate in neutral ammonium citrate (**NAC**) and water with bone ash percentage in chicks and young turkeys. The differences in availability of the P from various types of phosphates did not appear to be correlated with the solubility tests conducted, except that the more water-soluble compounds tended to be more available. Day et al. (1973) analyzed P solubility in 0.4% HCl, 2% citric acid (**CcA**), and NAC. The samples were also evaluated for biological availability by using bone ash. Comparing results on a group basis showed that acid solubility did not correspond to biological availability. The authors concluded that P solubility in dilute acids could not be used to predict bioavailability. In contrast, Yoshida and Hoshii (1979) observed a highly significant correlation between solubility in CcA solution and biological availability of P in chicks. The authors examined the relationship between solubility in a diluted CcA solution and biological availability of various inorganic calcium phosphates and P sources of animal origin. The solubilities of reagent grade mono-, di-, and tri-basic calcium phosphates in 0.5% aqueous CcA solution were 100%, 81.6%, and 54%, respectively. The biological availabilities were 126%, 114%, and 96%, respectively. The authors suggested an equation which allowed the estimation of a biological availability of P in samples of inorganic or animal origin from the solubility of the samples in the CcA solution.

Pensack (1974) determined the water-soluble P of different commercially available dicalcium und defluorinated phosphates. The author noticed that although the P in defluorinated phosphate (**DFP**) was not soluble in water, the chicks were able to utilize a major portion of the P contained therein. Gueguen (1994) pointed out that water solubility, which is not necessary for absorption, is a poor and unacceptable indicator of the availability of feed phosphates. Many phosphates that are insoluble in water are actually very good sources of P for animals (Gueguen, 1999). Sullivan et al. (1992) assessed the correlation of the biological value of feed phosphates with their solubility in water, diluted hydrogen chloride, CcA, and NAC. The correlation of the water solubility of phosphates to their relative bioavailability was low, and water solubility was a poor indicator of bioavailability. When 0.4% HCl was used, the correlation coefficients were 0.55, 0.33, and 0.72 for mono-

dicalcium phosphate, di-monocalcium phosphate, and DFP, respectively. The solubility of feed phosphates in 2.0% CcA or NAC was positive correlated with bioavailability (Table 3).

Table 3: The relative biological value (**RBV**) of feed phosphates and correlation to their solubility in water, 0.4% HCl, 2.0% CcA and NAC (Sullivan et al., 1992)

	Feed phosphate product		
	MDCP[2] (21% P)	DMCP[3] (18.5% P)	DFP (18% P)
RBV[1]	97.6	94.6	90.8
Solubility in water	67.5	38.8	8.9
Correlation to RBV	0.42	0.20	0.02
Solubility in 0.4% HCl	93.5	96.4	97.6
Correlation to RBV	0.55	0.33	0.72
Solubility in 2.0% CA	93.5	92.4	70.1
Correlation to RBV	0.30	0.95	0.92
Solubility in NAC	93.2	88.8	62.7
Correlation to RBV	0.46	0.87	0.93

[1] RBV as determined in a bioassay of 21 d duration with turkey poults; response criteria were tibia ash, weight gain and gain:feed ratio. Reference standard was calcium phosphate dibasic dihydrate.

[2] MDCP = mono-dicalcium phosphate.

[3] DMCP = di-monocalcium phosphate

The authors concluded that users could perform either a 2% CcA or a NAC solubility test as a screen for biological value along with other quality control procedures.

Coffey et al. (1994) evaluated the bioavailability of P in five defluorinated phosphates that differed in P solubility in NAC. A slight positive relationship existed between the NAC solubility of P and the bioavailability of P in DFP for chicks.

The solubility percentage of P supplements should not be confused with absorbability, because it is merely a simple means of classifying good, medium, and bad feed phosphates and is not discriminating enough to differentiate between high-quality phosphates (Gueguen, 1999). Ammerman (1995) pointed out that, generally, *in vitro* solubility was a poor indicator of *in vivo* bioavailability.

2.3. INFLUENCING FACTORS IN THE DETERMINATION OF P AVAILABILITY

The biological availability of P can be influenced by dietary factors such as the concentration of Ca, vitamin D, micro minerals, dietary energy in the diet; as well as physiological, health, and management factors (feed consumption, growth rate, sex, age, temperature, light program, etc.); choice of dietary ingredients, the availability of nutrients in these ingredients, and the

relationship between the concentration of other nutrients and P, as well as the type and concentration of P in the diet (Angel, 2006). Detailed reports of factors that affect the availability of P and other minerals were given by Gueguen (1961), Sullivan (1999), Jongbloed and Kemme (2002), and Kiarie and Nyachoty (2010). One important factor is diet composition. Mostly, either purified or practical-type diets have been used in experiments with poultry. Using the purified type of basal diet allows obtaining a very low initial P level which permits a wide range of P supplementation. It is also of advantage for sources with high P availabilities that can be more effectively evaluated with this diet than with some practical-type diets (Sullivan and Douglas, 1990). The very low P level in this type of diet requires the bird to obtain the major portion of its P requirement from the phosphate supplement (Wilcox et al., 1954). Thus, the supplemental phosphate may represent even more than 90% of the total P contained in the purified diet. The effect of anti-nutritive factors such as phytate can be avoided. Cornstarch, dried blood fibrin, gelatin, whey protein, potato protein, sucrose, cellulose, soybean oil, lard, amino acids, vitamins, and minerals have been used as the ingredients for the composition of purified basal diets. These ingredients, however, make this type of diet only poorly consumed by chicks and also question the usage of the obtained results for practical feeding.

Practical or similar-to-practical-type diets are mostly based on corn and soybean meal. Phytase activity in maize and soybean meal is very low and not of practical importance (Kornegay, 2001). The P level can be lowered by addition of ingredients with low P content such as cornstarch, potato protein (low ash quality), dried egg white, or gelatin. The supplemental phosphate may represent only the smaller portion of the total P contained in such a type of diet. However, the main ingredients used in these diets are easy to obtain, inexpensive and identical or similar to ingredients used in commercial broiler diets; also, these diets are more palatable to the broilers (Sullivan and Douglas, 1990).

It is still not clear what impact a supplemental inorganic phosphate may have on the PP hydrolysis in the digestive tract of broilers. Van der Klis and Versteegh (1996) showed under standard experimental conditions in 4-wk-old broilers that about 38% of IP_6 was degraded in peas and 69% in soybean meal. Increasing the dietary aP content (from 1.8 to 3 g/kg) by MCP caused a small reduction in IP_6 degradation (from 69% to 58% in soybean meal, $P < 0.05$; from 38% to 35% in peas, $P > 0.05$). Manangi and Coon (2008) demonstrated a decline of PP hydrolysis in a corn-soybean meal-based diet without phytase addition from 49.2% to 4.2% when nPP was increased from 0.08 to the NRC (1994) recommended level of 0.45%. The Ca concentration in the diets was maintained at 0.9%. However, if the Ca

concentration was only 0.5% in the test diets, no effect of nPP level on PP hydrolysis was observed. The hydrolysis and absorption of PP by monogastric animals are complex processes that are influenced by factors such as dietary Ca, inorganic P, vitamin D_3, age and type of birds, dietary ingredients, and feed processing (Sebastian et al., 1998).

2.4. WORK HYPOTESIS

Despite the great number of publications that are available on P nutrition in poultry, a gap in the knowledge of some important issues still exists. The literature has not provided answers yet to the question about the relationship among the various response criteria used in the evaluation of dietary P. Thus, in the first study of this thesis (Chapter 3) the objectives were (1) to compare the availability of two mineral phosphates determined with a regression approach based on either P retention or pc digestibility, and (2) to compare the measurements between 3-wk- and 5-wk-old broilers. In the second experiment (Chapter 4), which was linked to the first study, the objectives were (1) to determine the relationship between tibia bone ash and other bone criteria (2) in two age periods of broiler chickens, and (3) to compare the relationship between these response criteria with measurements of P retention. Suitability of tibia P retention for estimating whole body P retention in a current broiler strain was also assessed (Chapter 5). A big part of existing quantitative data on P availability for various feed phosphates given in the literature was determined using a purified type of diet. Thus, in a further experiment (Chapter 6) the objective was to determine the availability of a feed phosphate based on quantitative P retention by using a phytin-containing as well as a purified basal diet. The impact of the supplemental level of inorganic P on IP_6 hydrolysis was also evaluated.

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CHAPTER 3

COMPARISON OF RETENTION AND PRECECAL DIGESTIBILITY MEASUREMENTS IN EVALUATING MINERAL PHOSPHORUS SOURCES IN BROILERS[1,2]

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3. COMPARISON OF RETENTION AND PRECECAL DIGESTIBILITY MEASUREMENTS IN EVALUATING MINERAL PHOSPHORUS SOURCES IN BROILERS

3.1. ABSTRACT

The objectives of this study were to compare measurements of retention and prececal (pc) digestibility in evaluating mineral phosphorus (P) sources in 3- and 5-wk-old broilers. A corn-soybean meal-based BD was used (0.35% P on dry matter basis). Anhydrous monosodium phosphate (MSP_a) or anhydrous dibasic calcium phosphate (DCP_a) was supplemented to increment the P concentration by 0.08%, 0.16%, and 0.24%. Titanium dioxide was used as the indigestible marker. Diets were pelleted through a 3-mm screen. Two retention trials with excreta collection from d 16-20 and d 30-34 were conducted (n=8 birds per diet). Another 8 pens of 10 birds from the same hatch were allocated to each diet on d 11 or 25 each to measure pc digestibility in both age periods. After 10 d of feeding, these birds were asphyxiated by carbon dioxide exposure and the content of a defined section of the terminal ileum was obtained. Neither the source nor the level of P significantly affected P retention and pc digestibility of the diets. Percentage P retention and pc digestibility for MSP_a and DCP_a were calculated by linear regression analysis. In 3-wk-old broilers, P retention for MSP_a was 70% and significantly higher ($P < 0.001$) than for DCP_a (29%). Values determined for pc digestibility at the same age were very similar (67% for MSP_a and 30% for DCP_a; $P < 0.001$). In 5-wk-old broilers, P retention was 63% (MSP_a) and 29% (DCP_a) ($P < 0.001$), and pc digestibility was 54% (MSP_a) and 25% (DCP_a) ($P = 0.002$). We concluded that both retention and pc digestibility can be used for evaluating mineral P sources in broilers based on a regression approach. In 3-wk-old broilers, results obtained with both approaches were the same. In 5-wk-old broilers, the ranking of the two P sources was the same for both approaches. Values differed not greatly between the two age periods, but further studies on the relevance of broilers' age in P evaluation are suggested.

3.2. INTRODUCTION

Plant ingredients are the main basis for compound poultry feed. Phosphorus (**P**) in plant ingredients is largely present in the form of phytic acid and its salts (Eeckhout and de Paepe, 1994), and in this form only partly available to poultry. Mineral phosphates are therefore

widely used to increase the concentration of available P in the diet and meet the birds' requirement. Knowledge about the availability of P from mineral sources gained in importance since, on the one hand, the prices for feed phosphates sharply increased and on the other hand, environmental concerns associated with excessive excretion of P by livestock became greater. Thus, it is necessary to evaluate mineral P sources regarding their availability to poultry in order to meet their P requirements and to avoid excessive supply and P excretion. "Availability" in the context of this paper is a criterion that describes the potential of a P source. It is understood as that proportion of dietary total P that, at marginal level of P supply, can be utilized to cover the P requirements of an animal (Rodehutscord, 2009).

Different experimental techniques are in use to measure or estimate P availability (Rodehutscord, 2009). Quantitative measurement of P retention is one way. The experimental effort is high and balance cages are needed, but the measurements are precise and can be directly interpreted as P availability. Measurements of bone data, growth, or blood concentrations are in use to compare different P sources. Such relative bioavailability data always depend on the response determined for a reference P source; therefore, the choice of the reference source affects the results. For evaluation of feed proteins and amino acids, measurements of disappearance at the end of ileum are very common in broiler studies, because they exclude the effects of postileal fermentation (Ravindran et al., 1999). Such measurements of prececal (**pc**) digestibility applied to P may be an alternative to retention measurements. They do not depend on the use of balance cages. Probably more important is that the contribution of regulatory P excretion in the urine can be excluded. For this reason, pc measurements are the preferred approach for P evaluation in laying hens (van der Klis et al., 1997; Rodehutscord et al., 2002). It is not clear, however, whether measurements based on retention and pc digestibility deliver the same results when different mineral P sources are compared for their availability in broilers.

A small number of studies have examined either retention or pc digestibility of P from mineral sources, mostly at one certain age of birds and often using semipurified or purified types of diets (Ketels and De Groote, 1988; van der Klis and Versteegh, 1996; De Groote and Huyghebaert, 1997; Leske and Coon, 2002; Rodehutscord and Dieckmann, 2005). These studies have demonstrated that broilers utilize P from different mineral sources to a different extent than Pekin ducks do, for example (Wendt and Rodehutscord, 2004). However, published data are difficult to compare, because it is not clear to what extent details of the methodological approach affected the results and possibly masked real differences between the P sources. One of the relevant factors could be the age of birds. Rodehutscord et al. (2003)

did not find differences in the ability of Pekin ducks to utilize P from monobasic calcium phosphate between 3 and 5 weeks of age. To the best of our knowledge, the effect of age has not been studied in broilers yet.

Our objectives were therefore (1) to compare the availability of two mineral phosphates determined with a regression approach based on either P retention or pc digestibility, and (2) to compare the measurements between 3-wk- and 5-wk-old broilers. Two balance trials and two digestibility trials were conducted.

3.3. MATERIALS AND METHODS

3.3.1. ANIMALS AND MANAGEMENT

Unsexed broiler hatchlings (Ross 308) were obtained from a local hatchery (Brüterei Süd, Regenstauf, Germany) and randomly allocated, 11 per pen, to 112 floor pens (154 × 154 cm) bedded with pine shavings. The temperature in the animal house was gradually reduced from 35 to 27.5°C between d 1 and 7, from 27 to 24°C between d 8 and 14, and from 23.5 to 22°C between d 15 and 21, after which it remained constant. Water was always available from a nipple drinker and feed from a feeder trough. Before the trials started, chicks were provided free access to a broiler starter diet containing (per kg) 238 g CP, 12.5 MJ ME, 10.6 g of Ca, and 7.1 g of P (4.5 g non-phytin P (**nPP**)). A total of 20 hours of light were provided per day throughout the study. Mortality was recorded daily. The study was approved by the Animal Welfare Commissioner of the University in accordance with Animal Welfare Regulations.

3.3.2. EXPERIMENTAL DIETS

The diets were formulated to meet or exceed the requirements of the Gesellschaft für Ernährungsphysiologie (1999) for all nutrients except for Ca und P. Main ingredients such as corn, soybean meal, potato protein, and corn starch were chosen to achieve a low P concentration and low intrinsic phytase activity. Concentrations of Ca and P were calculated to be 0.7% and 0.35% in the basal diet (**BD**), on dry matter (**DM**) basis (Table 4).

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Table 4: Ingredient composition of the basal diet and analyzed concentrations

Ingredient composition (g/kg)	
Corn	522
Soybean meal, solvent extracted, 51 % CP	190
Potato protein, 75 % CP	129
Corn starch	85
Soybean oil	20
D,L-methionine	1.5
Mineral mix[1]	1.0
NaCl	1.0
Sodium bicarbonate	3.0
Vitamin mix[2]	1.5
Choline chloride	2.0
Titanium dioxide	5.0
Exchange mixture[3]	39.0
Analyzed concentrations (g/kg dry matter)	
Crude protein	255
Crude fat	60
Calcium	7.1
Phosphorus	3.5

[1] Mineral mix (Celita SG 1,GFT MBH, Memmingen, Germany) provided per kg of diet: Cu, 15 mg; I, 1.6 mg; Fe, 90 mg; Mn, 120 mg; Zn, 80 mg; Co, 0.6 mg; Se, 0.5 mg.

[2] Vitamin mix (Raiffeisen Kraftfutterwerke Süd GmbH, Würzburg, Germany) provided per kg of diet: vitamin A, 9.000 IU; vitamin D_3, 2.250 IU; vitamin E, 22.5 mg; menadione, 1.8 mg; thiamine, 2.3 mg; riboflavin, 4.5 mg; niacin, 37.5 mg; Ca-D-Pantothenate, 10.5 mg; pyridoxine, 4.5 mg; vitamin B_{12}, 23 µg; folic acid, 0.75 mg; biotin, 0.075 mg.

[3] Exchange mixture contained cellulose and sand (1:1) and limestone. In the six test diets this mixture was partially replaced by MSP_a or DCP_a, as detailed in the text. The limestone concentration was adjusted to maintain a Ca to P ratio in all diets of 2 to 1.

Anhydrous dibasic calcium phosphate (**DCP_a**; Sigma-Aldrich, Inc., Mo, USA) and anhydrous monosodium phosphate (**MSP_a**; Dr. Paul Lohmann GmbH KG, Emmerthal, Germany) were used as the two mineral P sources under test. Together with limestone,

inclusions of DCP_a and MSP_a were varied to achieve the targeted concentrations in another six diets. These test diets were calculated to contain 0.08%, 0.16%, or 0.24% of supplemental P from each of the two mineral P sources. P and Ca levels were adjusted by replacing cellulose and sand in the BD with respective levels of limestone, MSP_a, and DCP_a. The Ca concentration was increased up to 1.2% of dietary DM in a way that the ratio of Ca to P was similar in all diets. Titanium dioxide was included as indigestible marker at a rate of 0.5%. Diets were pelleted without steam through a 3-mm die. Samples of diets were collected immediately before the beginning of the trials. Samples were ground through a 0.5-mm sieve of a grinding mill (Type ZM 1, Retsch GmbH, Haan, Germany) and stored at room temperature to await analyses. Analyzed concentrations of Ca and P confirmed the intended levels (Table 5).

Table 5: Calculated and determined concentrations of total P and Ca in the experimental diets (g/kg DM)

	Total P		Ca	
	Calculated	Determined[1]	Calculated	Determined[1]
Basal diet	3.5	3.54 ± 0.03	7.1	7.13 ± 0.04
MSP_a (0.08 % P)	4.3	4.27 ± 0.01	8.6	9.78 ± 0.12
MSP_a (0.16 % P)	5.1	5.18 ± 0.09	10.2	10.78 ± 0.04
MSP_a (0.24 % P)	5.9	5.93 ± 0.04	11.8	12.79 ± 0.19
DCP_a (0.08 % P)	4.3	4.26 ± 0.05	8.6	8.16 ± 0.15
DCP_a (0.16 % P)	5.1	5.06 ± 0.07	10.2	9.51 ± 0.16
DCP_a (0.24 % P)	5.9	5.87 ± 0.07	11.8	11.59 ± 0.37

[1] Mean and standard deviation of three samples per diet (retention trial in Period 1 and Period 2, digestibility trial). Each analysis was run in duplicate.

3.3.3. RETENTION TRIALS

For the determination of P retention, 56 chicks with a BW of 240 ± 15 g (Period 1) and 1150 ± 50 g (Period 2) were used. Different birds were used in Period 2. Broilers were placed individually into balance cages (45 cm wide × 50 cm deep × 42.5 cm high) at d 11 (Period 1) and 25 (Period 2) of age. Birds were allocated at random to one of the seven experimental

diets (eight birds per diet). The balance cages were equipped with a trough and a drinking water beaker at the front side. Excreta were collected from trays underneath the wire mesh floor of the pens. Each retention trial consisted of a 4-d period of adaptation to the respective experimental diet and a 5-d main balance period with restricted feeding and complete excreta collection. Daily feed allowance was 50 g/bird (Period 1) and 100 g/bird (Period 2), which was approximately 90% of the ad libitum intake measured with birds that received the BD during the 3 d before the start of the collection period. The slight restriction in the amount of feed should ensure that differences in P intake originated only from the supplemented mineral P sources. However, feed intake was incomplete for some individuals in Period 1, and these refusals were recorded. Feed was offered in two meals per day at about 0800 and 1700 h. Excreta were collected for 5 d after the morning feeding from the tray underneath the pen. Excreta were bulk-sampled for each individual bird and stored at -20°C. Feathers were removed before each collection. Later, the excreta were defrosted, dried at 65°C in a convection oven (Heraeus UT 6760, Hanau, Germany), and ground as described for the feed above. Chicks were weighed individually on d 11 and d 20 (Period 1), and on d 25 and 34 (Period 2).

3.3.4. DIGESTIBILITY TRIALS

For the determination of pc digestibility, 560 chicks at d 11 and 25 of age each were weighed individually and distributed among 56 floor pens with 10 birds per pen to minimize variation in mean BW among pens. Broilers were from the same flock as those of the retention trials. Each pen was randomly assigned to one of the seven diets (eight replicated pens per diet). Feed and drinking water were freely available from one feeder and one nipple drinker per pen. Mortality was controlled on a daily basis. After 10 d of feeding the experimental diets, the birds were asphyxiated by CO_2 exposure. The abdominal cavity was immediately opened, the small intestine exposed, and the section between Meckel's diverticulum and 2 cm anterior to the ileo-ceca-colonic junction dissected. Only the terminal two-thirds of this section were used for digesta sampling (Rodehutscord et al., submitted to Poultry Science). The digesta was gently flushed out using double distilled water, pooled from all birds of one pen and frozen at -20 °C. Later, the digesta samples were freeze-dried (Type 102000, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany**)** and ground as described for the feed above. Individual BW was recorded at d 11 and 21 (Period 1) and at d 25 and 35 (Period 2) of age and the mean was calculated for each pen. Feed consumption was measured

on a pen basis. Total BW gain and feed efficiency were calculated with adjustment for mortality.

3.3.5. CHEMICAL ANALYSES AND CALCULATIONS

Samples of feed, excreta, and ileal digesta were analyzed for DM (103°C), P, Ca, and Ti. To determine the Ca, P, and Ti concentrations, a modification of method 10.6.1 (Verband Landwirtschaftlicher Untersuchungs- und Forschungsanstalten, 2006) was used for wet digestion. Duplicates of each sample were weighed (0.4 g) and transferred into glass digestion flasks. After adding 20 mL of sulfuric acid (95-97% (w/v), product no. 1650.2500, for analysis, NeoLab Migge GmbH, Heidelberg, Germany) and 2.5 mL of nitric acid (65% (w/v), product no. 865.2500, for analysis, Th. Geyer GmbH & Co. KG, Renningen, Germany), the flasks were heated from 100 to 200°C for 30 min in a block digestion system (Behr K 20 L, Behr Labor-Technik GmbH, Düsseldorf, Germany). After cooling, 2.5 mL of nitric acid were added and the solutions heated again from 225 to 300°C for 75 min. After return to room temperature, the solutions were transferred into 500-mL Erlenmeyer flasks, filled up with double distilled water, and filtered through paper filters (ashless MN 615 w, product no. 090304, Macherey-Nagel GmbH & Co. KG, Düren, Germany) into 100-mL plastic bottles. As suggested by Boguhn et al. (2009), Ti concentrations in the solutions were determined together with Ca and P concentrations using an inductively coupled plasma optical emission spectrometer (VISTA PRO, Varian Inc., Australia) and specific wavelengths for each element (Ca, 317.933; P, 213.618; and Ti, 334.941). Feed samples were also analyzed for CP (method 4.1.1) and crude fat (method 5.1.1) (Verband Landwirtschaftlicher Untersuchungs- und Forschungsanstalten, 2006).

The pc digestibility of P (y) was calculated for each diet on a pen basis according to the following equation:

$$y\ (\%) = 100 - 100 \times [(TiO_{2\ Diet} \times P_{\ Digesta}) / (TiO_{2\ Digesta} \times P_{\ Diet})]$$

where $TiO_{2\ Diet}$ and $TiO_{2\ Digesta}$ = analyzed concentrations of TiO_2 in the diet and digesta samples (g/kg), and P_{Diet} and $P_{Digesta}$ = analyzed concentrations of P in the diet and digesta samples (g/kg). The pc digestibility of Ca was calculated accordingly, using analyzed Ca concentrations. The retention of P and Ca was also calculated by using this equation, although feed intake and amount of excreta were quantified. This was done because we wanted to compare the two mineral P sources based on excreta and ileal digesta measurements and therefore intended to equalize the methodological approaches as much as possible.

The retention and pc digestibility of the two mineral P sources was obtained from the slope of linear regressions of the type y = a + mx, calculated between the level of added inorganic P (g/kg feed DM) (x) and the retained or pc digested amount of P (g/kg feed DM) (y). The calculated slope (m) multiplied by 100 is the percentage retention or pc digestibility of the supplemented P source. By this regression the response to the two P sources was separated from the P contained in theBD, and corrections for basal endogenous losses were not necessary. Calculations were made using data for the BD and the three levels of added P for each P source. Thus, the number of datasets used in regression analysis for each P source was 32. Regressions were calculated using GraphPad Prism 5.0 (Graph Pad Software, Inc.).

3.3.6. STATISTICAL DATA ANALYSES

The pen served as the experimental unit for statistical analyses in the digestibility trials and the individual bird was the experimental unit in the retention trials. ANOVA was performed to analyze data from each trial separately. All data were analyzed using the procedure for linear mixed models (PROC MIXED) of the software package SAS for Windows (version 9.1.3, SAS Institute Inc., Cary, NC, USA). The analysis was done in accordance with the model $y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + e_{ijk,}$ where y_{ijk} is the parameter, μ is the overall mean, α_i is the phosphate type (i = MSP_a and DCP_a), β_j is the level of phosphate, γ_{ij} is the interaction of phosphate type and level, and e_{ijk} is the error term. If necessary, heterogeneous variance was allowed for every combination of phosphate type and level.

3.4. RESULTS

3.4.1. RETENTION TRIALS

In Period 1, P retention for the BD was 54.5% (Table 6). No significant interaction between P source and supplementation level was detected. The addition of 0.08%, 0.16%, and 0.24% P from MSP_a to the BD resulted in P retention values of 59.0%, 61.8%, and 60.3%, respectively, without a significant effect of the P level. For 0.08%, 0.16%, and 0.24% P DCP_a supplemented diets, P retention values were 49.7%, 43.6%, and 45.1%, respectively, and again without a significant effect of the P level.

In Period 2, P retention of the BD was 49.2%. There was no significant interaction between P source and P supplementation level. The addition of 0.08%, 0.16%, and 0.24% P from MSP_a to the BD resulted in P retention values of 55.2%, 56.3%, and 54.9%, respectively. For 0.08%, 0.16%, and 0.24% P DCP_a supplemented diets, P retention values

were 45.7%, 41.3%, and 41.8%, respectively. For both P sources, the effect of the P level was not significant.

Ca retention was significantly improved in Period 1 ($P = 0.011$) and 2 ($P = 0.004$) when the P concentration was increased (Table 6). Ca retention for the BD was 23.3% and 15.1% in Period 1 and 2, respectively.

The relationship between P intake and retained P in Period 1 was linear (Figure 1, upper panel). Retention of P from MSP_a and DCP_a, determined by linear regression analysis, was 70% and 29%, respectively (Figure 1, upper panel). The difference between the slopes for MSP_a and DCP_a was significant ($P < 0.001$). In Period 2, the response was similar to that in Period 1 (Figure 2, upper panel). Retention of P from MSP_a and DCP_a was 63% and 29%, respectively. Again, the difference between the slopes for MSP_a and DCP_a was significant ($P < 0.001$).

Table 6: BW gain, feed intake and retention of P and Ca of broilers at different ages fed the low-P basal diet or diets supplemented with two different sources and levels of mineral P in the retention trials (n=8 broilers per treatment)

	BD[1]	MSP_a			DCP_a			Pooled	*P* (ANOVA)		
		0.08%	0.16%	0.24%	0.08%	0.16%	0.24%	SEM	P source (S)	P level (L)	LxS
Period 1											
BW gain d 11-20, g	253	277	257	275	253	239	261	11.3	0.519	0.771	0.689
Feed intake d 15-19, g	228	243	230	248	231	218	238	9.5	0.630	0.177	0.880
P retention[2], %	54.5	59.0	61.8	60.3	49.7	43.6	45.1	2.1	0.750	0.312	0.100
Ca retention[2], %	23.3	37.0	40.9	41.0	24.0	21.8	34.4	2.6	0.062	0.011	0.245
Period 2											
BW gain d 25-34, g	424	468	457	454	463	429	439	14.6	0.852	0.208	0.719
Feed intake d 29-33, g	489	495	497	495	495	492	494	2.4	0.818	0.850	0.998
P retention[2], %	49.2	55.2	56.3	54.9	45.7	41.3	41.8	1.3	0.856	0.110	0.190
Ca retention[2], %	15.1	34.6	32.0	40.4	15.9	21.9	27.4	2.8	0.085	0.004	0.319

[1] BD: Basal diet; MSP_a: Anhydrous monosodium phosphate; DCP_a: Anhydrous dibasic calcium phosphate.

[2] Retention of P and Ca is expressed in % of intake. Excreta were collected on d 16-20 and d 30-34, respectively, and retention was calculated based on the marker technique.

3.4.2. DIGESTIBILITY TRIALS

BW gain, feed consumption, and feed per gain ratio were significantly improved by dietary P level, but not by P source, in 11- to 21-d-old birds ($P < 0.004$, Table 7). In 25- to 35-d-old birds, no significant effects on feed intake and feed per gain ratio were detected. Both P source and P level tended ($P < 0.1$) to affect BW gain. A significant interaction ($P = 0.043$) indicated that the effect of P level on BW gain was greater for MSP_a than for DCP_a. Mortality for the birds fed the BD or MSP_a (0.08%, 0.16%, and 0.24%) and DCP_a (0.08%, 0.16%, and 0.24%) supplemented diets were 3.8, 6.3, 1.3, 6.3, 1.3, 2.5, and 2.5 in Period 1 and 0, 6.3, 2.5, 1.3, 0, 1.3, and 2.5% in Period 2, respectively. No effect of P level or P source was found.

In Period 1 and 2, the pc digestibility of P from the BD was 54.1% and 41.5%, respectively (Table 8). No significant interactions between P source and P supplementation level were detected. In both periods, the pc digestibility of P was not significantly affected by P level or P source. The pc digestibility of Ca from the BD was 63.8% and 47.4% in Period 1 and 2, respectively. P source and P level had a significant effect on pc digestibility of Ca in Period 1 ($P = 0.049$ and 0.002, respectively). In Period 2, there was a significant interaction ($P = 0.013$) between the level and the source of P for pc digestibility of Ca. The P level and P source alone also had a significant effect ($P < 0.007$) on this value.

The relationship between intake and pc digested P in Period 1 was linear (Figure 1, lower panel), and pc digestibility for MSP_a and DCP_a, determined by linear regression analysis, was 67% and 30%, respectively. The differences between the slopes for MSP_a and DCP_a were significant ($P < 0.001$). In Period 2, the response was similar to that in Period 1 (Figure 2, lower panel). Retention of P from MSP_a and DCP_a was 54% and 25%, respectively. Again, the differences between the slopes for MSP_a and DCP_a were significant ($P = 0.002$).

The differences between the slopes for MSP_a or DCP_a retention and pc digestibility in Period 1 (0.70 vs. 0.67 and 0.29 vs. 0.30, respectively), and Period 2 (0.63 vs. 0.54 and 0.29 vs. 0.25, respectively) were not significant ($P > 0.05$).

Table 7: Body weight gain, feed intake and feed per gain ratio of broilers at different ages fed the low-P basal diet or diets supplemented with two different sources and levels of mineral P (n=8 pens of 10 birds per pen)

	BD[1]	MSP_a			DCP_a			Pooled	*P* (ANOVA)		
		0.08%	0.16%	0.24%	0.08%	0.16%	0.24%	SEM	P source (S)	P level (L)	LxS
d 11 to 21											
BW gain, g	342	367	430	427	388	394	439	13.4	0.748	<0.001	0.738
Feed intake, g	512	511	574	568	564	579	607	16.7	0.472	0.004	0.670
Feed/BW gain, g/g	1.50	1.39	1.33	1.34	1.46	1.44	1.38	0.02	0.386	0.004	0.720
d 25 to 35											
BW gain, g	832	837	888	925	877	870	867	23.7	0.053	0.099	0.043
Feed intake, g	1421	1413	1484	1477	1475	1482	1457	25.6	0.101	0.373	0.115
Feed/BW gain, g/g	1.71	1.69	1.68	1.61	1.69	1.71	1.68	0.03	0.471	0.161	0.391

[1] BD: Basal diet; MSP_a: Anhydrous monosodium phosphate; DCP_a: Anhydrous dibasic calcium phosphate.

Table 8: Prececal (pc) digestibility of P and Ca at different ages of broilers fed the low-P basal diet or diets supplemented with two different sources and levels of mineral P (n=8 pens of 10 birds per pen)

	BD[1]	MSP_a			DCP_a			Pooled	*P* (ANOVA)		
		0.08%	0.16%	0.24%	0.08%	0.16%	0.24%	SEM	P source (S)	P level (L)	LxS
Period 1											
pc P digestibility, %	54.1	55.1	60.8	58.2	45.7	46.3	43.7	1.4	0.730	0.705	0.093
pc Ca digestibility, %	63.8	62.9	59.1	55.6	53.7	50.6	50.6	1.6	0.049	0.002	0.199
Period 2											
pc P digestibility, %	41.5	48.8	47.5	47.2	39.2	35.9	35.1	2.4	0.839	0.361	0.694
pc Ca digestibility, %	47.4	58.8	58.7	35.0	45.9	37.5	35.1	2.7	0.007	<0.001	0.013

[1] BD: Basal diet; MSP_a: Anhydrous monosodium phosphate; DCP_a: Anhydrous dibasic calcium phosphate.

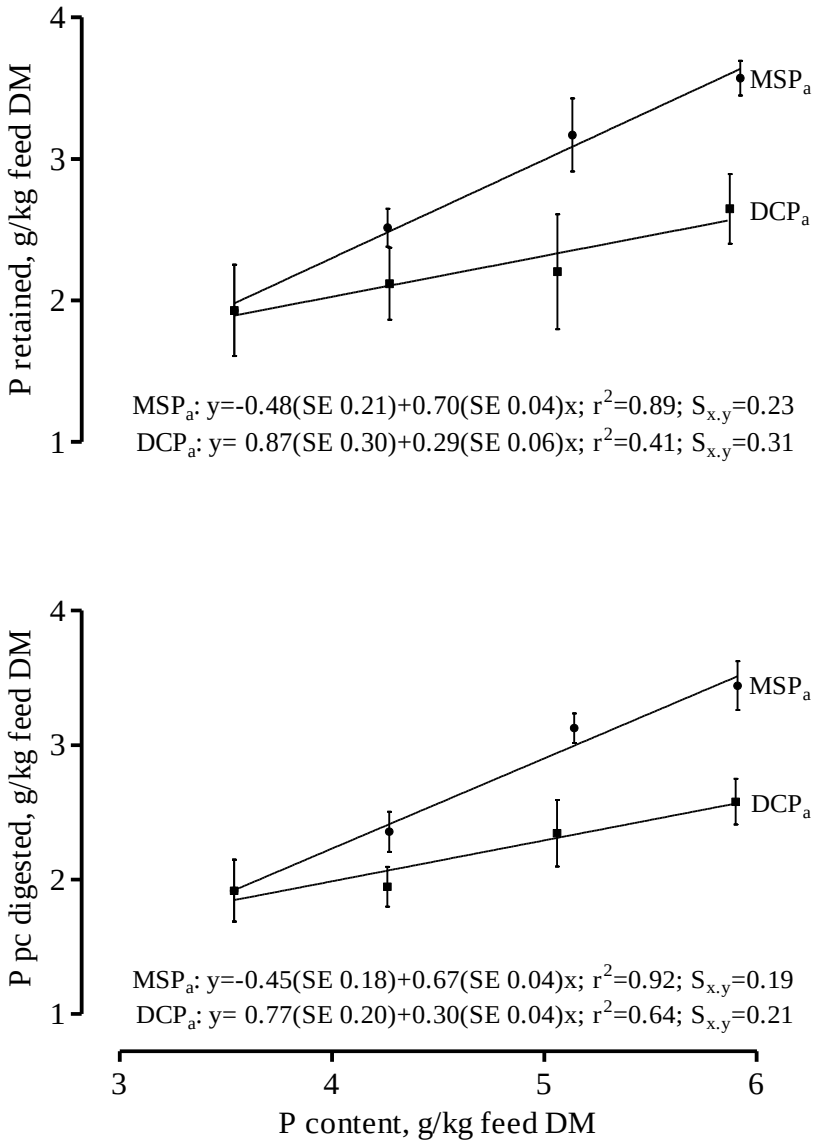

Figure 1. P retention (upper panel) and P pc digestibility (lower panel) depending on P content of diet in Period 1 (mean and SD; n=8 replicates per treatment)

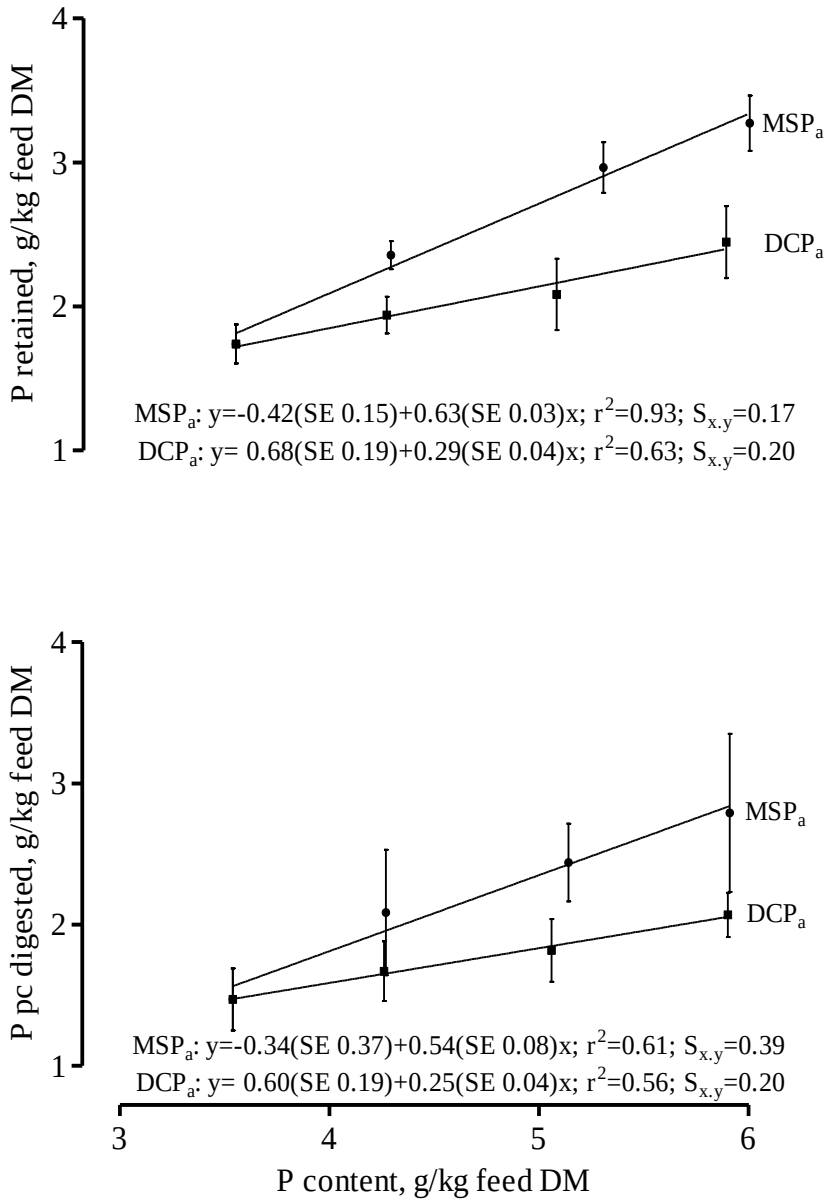

Figure 2. P retention (upper panel) and p pc digestibility (lower panel) depending on p content of diet in Period 2 (mean and SD; n=8 replicates per treatment)

3.5. DISCUSSION

3.5.1. EXPERIMENTAL APPROACH

According to Eeckhout and De Paepe (1997), regression analysis is the most convenient approach for comparisons, because the ratio of the calculated slopes allows for a direct comparison of the phosphates involved. It is also possible to compare different criteria of P evaluation using the slopes of linear regressions. The results of our experiment confirm that P from the two mineral sources has different availabilities, with the higher one for MSP_a in both age periods. The slopes for MSP_a (0.67) and DCP_a (0.30) pc digestibility in Period 1 had a ratio of 2.23, which was close to the slope ratio (2.41) determined for P retention (0.70 and 0.29, respectively). The differences between the slopes for retention and pc digestibility for either MSP_a or DCP_a in Period 1 (0.70 vs. 0.67 and 0.29 vs. 0.30, respectively) were not significant (P = 0.61 and 0.81, respectively). Hence, a common slope for both datasets within each P source could be calculated, and the determined pooled slopes in Period 1 were 0.68 (MSP_a) and 0.29 (DCP_a).

Differences between pc digestibility and retention of P could occur as a consequence of either P excretion with urine or postileal absorption and secretion of P. Manangi and Coon (2006) used 40- and 50-d-old colostomized broilers to estimate the effect of different dietary nPP levels on urinary P excretion. They found that urinary excretion of P remained constant and very low from 0.08% to 0.28% dietary nPP in 40-d-old birds (6.0±3.2 mg/d), and 0.08% to 0.21% dietary nPP in 50-d-old birds (1.9±3.5 mg/d). Supplements above these thresholds caused an increase in urinary P excretion and indicated P supply above the requirements. In our study, the maximum increase in dietary nPP was 0.33%, which was higher than the threshold levels determined by Manangi and Coon (2006). However, broilers in our studies were younger and thus required a higher P concentration in the diet. Further, they were fed at a restricted rate in the retention trials. If the urine had been relevant for P excretion, then P retention should have been lower than pc digestibility, which was not the case. Therefore, we assume that the threshold for urinary P excretion was not exceeded and urinary P losses remained negligibly low. In non-ruminants, absorption of Ca and P occurs primarily in the small intestine (Veum, 2010). Hurwitz and Bar (1970) reported for 3-wk-old chicks that most of the absorption of Ca and P occurred in the jejunum. No absorption of Ca and P was noted posterior to the upper ileum. Similar results were obtained for Ca by van der Klis et al. (1990) who studied absorption of minerals in the entire gastrointestinal tract in 6-wk-old broilers. Thus, although not measured, there is good evidence to assume that in our study none or only

very little P absorption occurred in the section of the lower ileum that we took samples from. As further indirect evidence, the study of Biehl and Baker (1997) can be taken. They found no differences in tibia ash between cecectomized and intact chicks. This suggests that inorganic phosphate, although released from inositol phosphates by microbial activity in the ceca (Kerr et al. 2000), was not absorbed. Based hereupon, we can conclude that postileal absorption had no relevance in our study. However, it is still possible that the similarity of data for P retention and pc digestibility for both mineral P sources was the consequence of a compensation of postileal absorption by urinary excretion. Whether such compensation was relevant or not cannot be evaluated on the basis of the present study.

The results in Period 2 were similar to that in Period 1. The slope ratio for MSP_a and DCP_a pc digestibility (2.16) again was very close to the P retention slope ratio of 2.17. However, the percentage values of MSP_a and DCP_a retention were on a somewhat higher level than for pc P digestibility. This may have had different reasons. First, feed intake in the digestibility trial was by a factor of about 1.4 higher than in the retention trial, whereas this difference was less than 1.2 in Period 1. Consumption of P from both the BD and supplemented P sources therefore was higher, which might have brought P supply close to the level of requirement in the digestibility but not in the retention trial. Furthermore, the higher intake and digesta passage may have affected the site of absorption in the small intestine. Kluth et al. (2005) hypothesized that the large amount of material passing through the small intestine required a longer time to complete digestion of proteins, thus leading to differences in pc digestibility of amino acids when measured in different subsections of the ileum. Perhaps also in the present study, P absorption was not completed at the beginning of the section that was sampled. Second, it was suggested by Miyazawa and Yoshida (1991), based on rat studies, that an increased rate of digesta passage through the intestine might be relevant to the inhibitory effect of dietary inorganic P on phytate hydrolysis, since inorganic phosphate salts have an osmotic effect in the intestinal lumen. Their study and those of Moore and Veum (1982), Moore and Veum (1983), and Moore et al. (1984) showed by analysis of feces the inhibitory effect of supplemental inorganic P in the diets on phytate breakdown. And third, Ca has the capacity to interact with inorganic P and phytate in the gut lumen. Because the Ca to phytate P ratio increased along with the increment in supplemental P in our study, this could have led either to a flocculent precipitation of calcium orthophosphate or to the formation of Ca phytate complexes (Selle et al., 2009), which should have been more pronounced by the higher feed and P intake and might have affected pc digestibility of P.

3.5.2. AGE EFFECTS

P retention and pc digestibility for MSP_a as well as pc digestibility for DCP_a tended to be lower in 5-wk-old compared to 3-wk-old birds. Yan et al. (2005) reported a decrease in pc digestibility of P in broilers fed a low-P diet (0.6% Ca and 0.30% nPP) from 57 % at 23 d to 47% at 32 d of age. Simultaneously, the hydrolysis of phytin decreased from 40% to 16%. P retention and pc digestibility of the BD in the present study also declined from 54% (Period 1) to 49% (Period 2) and from 54% (Period 1) to 42% (Period 2), respectively. In contrast, Edwards et al. (1989) reported an increase in phytin P utilization from 19% to 36% with chicks that were 7 and 21 d old. This discrepancy found between studies may have been caused by other interfering factors, because level of Ca, nPP, or vitamin D3, as well as feed processing and feed or ingredient particle size can also cause variation in phytin hydrolysis (Angel et al. 2002). The relevance of changes in phytin hydrolysis is therefore difficult to judge.

The level of P supply in relation to the requirements may also have been relevant. Broilers used in Period 2 were raised on a starter diet with adequate Ca and P content and assigned to the experimental diets on d 25. Growing chicks after three weeks of age are less sensitive to dietary P deprivation; not only the feed consumption after three weeks of age increases considerably and with it the P intake, but also periods of rapid bone formation (4 to 18 days of age) and mineralization (4 to 11 days of age) pass off (Williams et al., 2000). Since in growing chicks 80% of P is essentially transferred to the skeleton (De Groote and Huyghebaert, 1997) after the third week post hatch, the fat content of the body and bones increases and the P need declines. Leske and Coon (2002) also reported that the lower dietary percentage of retainable P requirements for the grower period (21 to 42 d of age) was most likely due to the increase in feed consumption and a slowing of skeletal growth compared to the fast development during the starter period. The highest nPP level in the present study (3.3 g nPP per kg diet) was still below the NRC (1994) recommendation level of 3.5 g nPP for 3- to 6-wk-old broilers. Nevertheless, there is no final answer to the question whether the trend toward lower P retention and pc digestibility values was caused by a change in phytin hydrolysis or slightly excessive P intake in relation to the requirements. Pekin ducks did not differ in the utilization of a mineral P source when they were 3- or 5-wk-old (Rodehutscord et al., 2003).

3.5.3. MINERAL P SOURCE

Our retention and pc digestibility values obtained by linear regression analysis were lower than those reported in the literature. Van der Klis and Versteegh (1996) reported retention values of 92% for MSP×H_2O and 55% for DCP_a in 3-wk-old broilers. Ketels and De Groote (1988) reported pc digestibility of P from DCP_a and DCP×H_2O at 3 wk of age to be 67% and 73%, respectively. The discrepancies between our and these two studies can be attributed to differences in the hydration state (for MSP), experimental conditions or methods. The hydrous MSP has a higher P availability than its anhydrous form (De Groote and Lippens, 2002). Hydrated DCP also has higher availability than its anhydrous form in broilers (Ketels and De Groote, 1988; van der Klis and Versteegh, 1996; De Groote and Huyghebaert, 1997). Van der Klis and Versteegh (1996) used a purified BD (0.2 g P/kg) for their investigations. The experimental diets were standardized at 1.8 g available P/kg and 5 g Ca/kg. Ketels and De Groote (1988) used a BD that also was very low in available P. In the present study, we used a corn-soybean meal-based diet (0.35% P of feed DM), which was supplemented with three graded P levels. In young turkeys, Grimbergen et al. (1985) determined pc digestibility of P from DCP_a to be 35%, which confirms the values found in the present study with broilers. Leske and Coon (2002) demonstrated that the retention of P from different P sources depends on the amount of the P source included in a corn-soybean meal-based diet. In their study, P retention from a reagent-grade monocalcium phosphate declined from 98% to 59% when nPP was increased from 0.16% to 0.45%. The same tendency was observed in the present study. For instance in Period 1, MSP_a retention values were 81%, 78%, and 70% calculated by separate regression for only one (0.08%), two (0.08% and 0.16%) or three (0.08%, 0.16%, and 0.24%) inclusion levels, respectively. Wasserman and Taylor (1973) showed that with increasing concentration of P the rate of ^{32}P absorption from specific ligated intestinal segment tended to decrease. The authors suggested the existence of a saturable component in P absorption.

Using linear regression analysis for assessing the availability of a supplement is generally assumed to avoid possible interactions between the BD and a supplement. However, this may be different in P supplementation studies where supplemented mineral P can influence phytin hydrolysis from the BD, as shown in broilers (van der Klis and Versteegh, 1996; Manangi and Coon, 2006) and in rats (Moore and Veum, 1982; Moore and Veum, 1983; Moore et al., 1984; Miyazawa and Yoshida, 1991), and thus the calculated P retention or pc digestibility values of the mineral phosphate source. The usage of synthetic phytate-free diets in the evaluation of P availability allows eliminating such possible interaction, but this

implies the risk that data are not relevant to the poultry industry where phytate containing diets are widely used. Kornegay (2001) estimated that the retention of P from several feed-grade P sources was on average 46% for broilers and turkeys, which is much lower than the values obtained with purified diets by suboptimal P supply under standardized conditions (Van der Klis and Versteegh, 1996; De Groote and Huyghebaert, 1997).

We conclude that both retention and pc digestibility are suitable for assessing the availability of mineral phosphate sources in broilers. The ranking of phosphates is the same based on both approaches. Comparison of results with P sources studied herein and values from the literature suggest that methodological details have a great effect, and further standardization of the protocol is needed in order to achieve better comparability of results. Broilers at the age of 3 and 5 weeks do not greatly differ in their P availability values, but further studies on this aspect also including broilers that are younger than 3 wk are suggested.

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CHAPTER 4

COMPARISON AND EVALUATION OF BONE MEASUREMENTS FOR THE ASSESSMENT OF MINERAL PHOSPHORUS SOURCES IN BROILERS[1,2]

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4. COMPARISON AND EVALUATION OF BONE MEASUREMENTS FOR THE ASSESSMENT OF MINERAL PHOSPHORUS SOURCES IN BROILERS

4.1. ABSTRACT

The main objective of this study was to compare different bone measurements in regard to responses to supplements of mineral P sources. Comparisons were also made with P retention and digestibility responses determined in a companion study and with blood inorganic phosphate (P_i) responses. A corn-soybean meal-based basal diet was used (0.31% P). Anhydrous monosodium phosphate (MSP_a) or anhydrous dibasic calcium phosphate (DCP_a) was supplemented to increment the P concentration by 0.08%, 0.16%, and 0.24%. Each of the 7 diets was fed for 10 days starting 11 d (Period 1) or 25 d post hatch (Period 2). Tibia and foot (tarsometatarsus and toes) were dissected from 4 broilers per pen (8 pens per diet) and cleaned from adhering tissues. Bone ash and P were determined, and density criteria were measured using quantitative computed tomography. Responses were evaluated and compared based on linear regression analysis. In general, responses to MSP_a had a greater slope than DCP_a for all criteria studied. In Period 1, differences between the slopes were significant ($P < 0.05$) for almost all bone criteria. In Period 2, the slopes significantly differed for the amounts of ash and P of all bones studied, for tibia, tarsometatarus and foot ash percentage, for total and cortical density of tibiae, but not for the other criteria. For the different bones, the ratio of slopes for MSP_a and DCP_a was very similar based on the amount of ash in both periods. Foot ash proved to be as sensitive as tibia ash in both periods. The relationship between corticalis content based on quantitative computed tomography measurements and the amount of tibia ash was high ($R^2 = 0.94$) in Period 1. Blood serum P_i and BW gain were not suitable for P evaluation. We concluded that the ranking of both mineral P sources based on bone criteria differed from the ranking that was based on P retention or prececal digestibility. This underlines the need for developing a standard protocol of determination of available P in poultry.

4.2. INTRODUCTION

Avoiding phosphorus (**P**) deficiencies is very important for an optimal growth and bone development in broiler chicks. Bone formation during growth and bone remodeling both require input of calcium (**Ca**) and P for the new bone tissue formed (Williams et al., 2000).

Optimizing the P concentration in the feed also is a matter of feeding costs and reducing the impact on the environment. Avian species are able to utilize phytate P from plant feedstuffs only incompletely. Although more than half of the pig and poultry diets on global scale contain an exogenous phytase for enhancing the digestibility of phytate P and permitting lower dietary P levels (Selle et al., 2009), feed phosphates are still needed.

Because mineral P sources substantially contribute to feeding cost and feedstuff P availability is variable, knowledge of P availability is critical to efficient animal production (Soares, 1995). The question remains what the appropriate response criterion to measure feedstuff P availability is. Various approaches are in use by different laboratories for the determination of P availability (Rodehutscord, 2009). In growing birds, different bone measurements are considered as good criteria for estimating P availability, because about 80% of total P retention is in the skeleton (De Groote and Huyghebaert, 1997), mainly in the form of hydroxyapatite (Breves and Schröder, 1991). Many experiments have been performed to define the relative biological availability or value of mineral phosphate sources using bone ash or body weight gain as response criteria (Gillis et al., 1954; Nelson and Peeler, 1961; Ammerman et al., 1961; Nelson and Walker, 1964; Yoshida and Hoshii, 1977; Soares et al., 1978; Huyghebaert et al., 1980; Nelson et al., 1990; Ravindran et al., 1995; Lima et al., 1997; Garcia and Dale, 2006). In these studies, different bone criteria were used. Gillis et al. (1954) already used the tibia ash content as a reference for quantifying the availability of P for chicks. The method was based on relating percentage tibia ash from chicks fed a certain test supplement to that of chicks fed a beta-tricalcium phosphate. In subsequent decades, the search for precise, accurate, and rapid assays, which could be able to replace bone ash in P evaluation, continued. Akpe et al. (1987) examined the relative precision of single photon absorptiometry and bone ash methodologies as response criteria in the measurement of bioavailability of P from various supplements for turkeys. Onyango et al. (2003) used dual-energy X-ray absorptiometry (**DEXA**) with 3-wk-old broilers. The authors concluded that bone mineral content and density might be used to predict percentage tibia ash in broilers. However, there is only little information about the relationship between the Quantitative Computed Tomography (**QCT**) measurements and bone ash in broilers (Saunders-Blades et al., 2003). Blood inorganic P ($\mathbf{P_i}$) concentration has also been used in P evaluation (Gardiner, 1962; Hurwitz, 1964; Lima et al., 1997; Fernandes et al., 1999).

However, growth, bone data, or blood criteria, can express P availability only on a relative basis and are not of a quantitative nature (Peeler, 1972). While these assays are useful

for comparing different sources of Ca and P, their usefulness in formulating diets is limited because they do not provide biological retention values (Coon et al., 2002).

In spite of the large number of experiments carried out using different P sources, there is only little information about the relationship between different criteria in assessing P availability (Ravindran et al., 1995; Denbow et al., 1995; Yan et al., 2005). Methodological details and experimental conditions can substantially impact the results (Rodehutscord, 2009). Additionally, it is not clear in what relationship relative and quantitative criteria of P evaluation stand to each other.

The objectives of this study were therefore (1) to determine the relationship between tibia bone ash and other bone criteria two age periods of broiler chickens, and (2) to compare the relationship between these response criteria with measurements made on whole body P retention.

4.3. MATERIALS AND METHODS

The study comprised two periods with birds of different ages, but from the same hatch. Two different mineral phosphate sources with 3 supplemental levels each were used. The study was linked to a digestibility and retention study reported by Shastak et al. (submitted to Poultry Science).[3] Therefore, only the specific details are described here, while the reader is referred to Shastak et al. (submitted to Poultry Science) for a more detailed description of the diets and birds' management.

4.3.1. ANIMALS, MANAGEMENT AND EXPERIMENTAL DIETS

Unsexed broiler chicks (Ross 308) were obtained from a hatchery (Brüterei Süd, Regenstauf, Germany). Before the trials started, chicks were provided free access to a broiler starter diet. In the experimental phase, a corn-soybean meal-based basal diet (**BD**) was used (0.35% total P on dry matter (**DM**) basis). Anhydrous monosodium phosphate (**MSP_a**) or anhydrous dibasic calcium phosphate (**DCP_a**) was supplemented to increment the P content by 0.08%, 0.16%, and 0.24% on DM basis in another 6 diets (Table 9). Diets were pelleted without steam through a 3-mm die.

[3] The paper is attached to this submission as supplemental material. It still is under review.

Table 9. Calculated and determined concentrations of total P and Ca in the experimental diets (g/kg dry matter)[1]

	Total P		Ca	
	Calculated	Determined	Calculated	Determined
Basal diet	3.5	3.54	7.1	7.13
MSP_a (0.08 % P)	4.3	4.27	8.6	9.78
MSP_a (0.16 % P)	5.1	5.18	10.2	10.78
MSP_a (0.24 % P)	5.9	5.93	11.8	12.79
DCP_a (0.08 % P)	4.3	4.26	8.6	8.16
DCP_a (0.16 % P)	5.1	5.06	10.2	9.51
DCP_a (0.24 % P)	5.9	5.87	11.8	11.59

[1] Originally presented by Shastak et al. (submitted to Poultry Science). MSP_a: Anhydrous monosodium phosphate; DCP_a: Anhydrous dibasic calcium phosphate.

Five hundred and sixty chicks each on d 11 (Period 1) and 25 (Period 2) of age were weighed individually and distributed among 56 floor pens with 10 birds per pen in a way that variation in mean BW among pens was minimized. Each pen was randomly assigned to 1 of the 7 diets with 8 replicated pens per diet. Feed was available for *ad libitum* intake from one feeder per pen. Drinking water was also freely available from one nipple drinker per pen. Mortality was controlled on a daily basis. After 10 d of feeding the experimental diets, the birds were asphyxiated with CO_2. Four chicks, chosen to represent the average BW of birds in each pen, were taken for bone and blood sampling.

4.3.2. BLOOD SAMPLING AND ANALYSIS

The blood samples (5 mL) were taken immediately after asphyxiation from the jugular vein using 10-mL tubes. Following coagulation, samples were centrifuged for 15 min at 1940 *g* (Megafuge 2.0 R, Heraeus Sepatech GmbH, Germany). The supernatant serum was stored at 4°C for about 12 h until analyses of Ca and P_i concentrations. The serum was analyzed using the P module of Roche/Hitachi Modular Analytic System at the Veterinary Medical Laboratory (Ludwigsburg, Germany). The method of P_i determination was based on the reaction of phosphate with ammonium molybdate to form an ammonium phosphomolybdate complex $(NH_4)_3[PO_4(MoO_3)_{12}]$ in the presence of sulfuric acid without reduction. The

complex was determined photometrically in the ultraviolet region using a wavelength of 340 nm. Ca determination was based on the reaction of Ca with o-cresolphthalein complexone in an alkaline solution. Mg was masked with 8-hydroxyquinoline. The color intensity of the purple complex (Ca-o-cresolphthalein) formed is directly proportional to the Ca concentration and was measured photometrically.

4.3.3. BONE PREPARATION

The right tibiotarsus (tibia) and the right foot (tarsometatarsus together with all toes) were removed after slaughter, single packed in polyethylene bags, and stored at -20°C. Following defrosting of tibiae, most of the adhering soft tissues were manually removed, and the tibiae were then kept for 12 h (Period 1) and 16 h (Period 2) in a 9% Biozym SE solution. The pure Biozym SE (Spinnrad Inc., Norderstedt, Germany) contained 15-30% nonionic tensides, 5-15% alcohol denat, and 5% enzymes (amylase, protease). Then the leftovers of the connective tissues were removed by mild brushing. Bones were subsequently rinsed in distilled water and dried at 30°C for 48 h in a convection oven (Heraeus UT 6760, Hanau, Germany).

The defrosted feet were manually cleaned from the skin and kept in the 9% Biozym SE solution as described for the tibiae. Soft tissue leftovers were then removed, and tarsometatarsus and toe bones were separately rinsed in distilled water and dried as described for tibiae. The toe included all the *phalanges proximales et intermediae* and *Os metatarsale I.*

4.3.4. MEASUREMENTS

QCT measurements were carried out at half of the tibia length (± 0.5 mm) using the single-energy peripheral Stratec XCT 960 bone scanner (Stratec Medizintechnik GmbH, Pforzheim, Germany). The calibration was carried out with phantoms of a specified hydroxylapatite concentration. The total mineral density (**TD**) (mg/cm^3), the cortical plus subcortical mineral density (**CD**) (mg/cm^3), and the Polar Strain Strength Index (**SSI**) (mm^3) were calculated by the Stratec software package. The determination of the SSI or the stability of the bone towards bending or torsion was based on the calculation of the cross-sectional moment of inertia. Division of the cross-sectional moment of inertia by the maximum distance of any voxel from the center of gravity yielded the section modulus which is directly proportional to maximum stress in the bone. The cortical content (**CC**) (mg/mm) was calculated from the cortical area and cortical density.

Breaking strength of tibiae in Period 2 was determined using an Instron testing machine (Model 5565 with Bluehill-Software, Instron Deutschland GmbH, Pfungstadt, Germany). The tibia bone was held by two supporters (spaced 50 mm apart) and the physical power was applied to the midpoint of the bone by a static load cell (5 kN) with a crosshead speed of 200 mm/min. Tibiae obtained from birds in Period 1 were too short to be measured with this technique.

The tibia, tarsometatarsus, and toe samples were dried for 24 h at 105°C, weighed, ashed in a muffle furnace (Nabertherm L 40/11, Nabertherm GmbH, Bremen, Germany) at 600°C for 24 h, cooled in a desiccator, and weighed again. Tibia ash, tarsometatarsus ash, and toe ash were determined for individual bones.

Samples of the bone ash were transferred into glass digestion flasks. After adding 25 mL nitric acid (65% (w/v), product no. 865.2500, for analysis, Th. Geyer GmbH & Co. KG, Renningen, Germany), the flasks were heated at 100°C for 40 min on a hotplate (block digestion system Behr K 20 L, Behr Labor Technik GmbH, Düsseldorf, Germany). After cooling, 50 mL of double distilled water was added; the solutions were mixed, transferred into a 500-mL Erlenmeyer flask, and filled up with double distilled water. Concentrations of Ca and P were measured at specific wavelengths for each element (Ca, 317.933; P, 213.618) by using an Inductively Coupled Plasma Emission Spectrometer (VISTA PRO, Varian Inc., Australia).

4.3.5. CALCULATIONS

The slopes of linear regressions of the type $y = a + mx$ were calculated between the level of added mineral P (g/kg feed DM) and a given criterion. These calculations were made using data for the BD and three levels of added P for each of the two P sources. Hence, the number of datasets used for each P source was 32. Regressions were calculated using GraphPad Prism 5.0 (Graph Pad Software, Inc.).

4.3.6. STATISTICAL ANALYSIS

The pen (mean of 4 observations per pen) served as the experimental unit for all statistical analyses. ANOVA was performed to analyze data from each period. All data were analyzed using the procedure for linear mixed models (PROC MIXED) of the software package SAS for Windows (version 9.1.3, SAS Institute Inc., Cary, NC, USA). The analysis was in accordance with the following model:

$y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + e_{ijk}$

where y_{ijk} is the parameter, μ is the overall mean, α_i is the phosphate type (i = MSP_a and DCP_a), β_j is the level of phosphate, γ_{ij} is the interaction of phosphate type and level, and e_{ijk} is the error term. If necessary, heterogeneous variance was allowed for every combination of P source and level.

4.4. RESULTS

Data for BW and feed consumption were reported in detail before (Shastak et al., submitted to Poultry Science). BW gain, feed consumption, and feed per gain ratio were significantly improved by dietary P level, but not by P source, in 11- to 21-d-old birds ($P \leq 0.004$). In 25- to 35-d-old birds, no significant effects on BW gain, feed intake and feed per gain ratio were detected.

4.4.1. BONE CHARAKTERISTICS

In Period 1, significant interactions between the level and the source of P were found for concentrations and amounts of ash in different bones, except for toe ash concentration ($P = 0.111$) (Table 10). The P source had a significant effect on the amount of ash, but not on ash concentrations. With supplementation of MSP_a, ash amount of bones was significantly higher than with supplementation of DCP_a. The effect of level of P was highly significant ($P < 0.001$) for both concentrations and amounts of ash in all bones. In Period 2, the level of P supplementation also had a significant effect on tibia ash and tarsometatarsus ash concentrations and amounts ($P \leq 0.001$) (Table 10). The toe ash concentration was not significantly affected by the P level. No significant effect of the P source was found in Period 2. A significant interaction ($P < 0.05$) was determined between the level and the source of P for the ash amounts of tibia, tarsometatarsus, toe, and foot.

Table 10. Concentration and amount of ash determined in bones of broilers at different ages following feeding diets with different inclusion levels of two different mineral P sources

	BD[1]	MSP_a			DCP_a			Pooled	*P* (ANOVA)		
		0.08%	0.16%	0.24%	0.08%	0.16%	0.24%	SEM	P source (S)	P level (L)	LxS
Period 1											
Tibia ash (%)	41.5	44.3	48.3	51.2	43.7	45.4	47.1	0.83	0.128	<0.001	0.027
Tibia ash (g/bone)	0.29	0.37	0.50	0.60	0.36	0.42	0.48	0.01	<0.001	<0.001	<0.001
Tarsometatarsus ash (%)	34.3	39.5	42.9	46.1	38.1	40.4	41.3	0.82	0.137	<0.001	0.042
Tarsometatarsus ash	0.15	0.20	0.28	0.34	0.19	0.23	0.26	0.01	<0.001	<0.001	<0.001
Toe ash (%)	45.2	47.5	48.4	50.2	47.2	47.9	48.2	0.54	0.185	<0.001	0.111
Toe ash (mg)	68	88	123	155	82	101	118	2.97	<0.001	<0.001	<0.001
Foot ash[2] (g)	0.22	0.29	0.40	0.50	0.27	0.33	0.38	0.01	<0.001	<0.001	<0.001
Period 2											
Tibia ash (%)	41.6	44.2	47.5	48.4	43.1	44.3	45.2	0.53	0.246	<0.001	0.061
Tibia ash (g/bone)	1.53	1.84	2.08	2.21	1.74	1.85	1.87	0.05	0.104	<0.001	0.022
Tarsometatarsus ash (%)	38.4	40.4	42.8	44.2	38.9	40.2	40.2	0.74	0.277	0.001	0.100
Tarsometatarsus ash	0.79	0.95	1.09	1.18	0.88	0.95	0.97	0.03	0.133	<0.001	0.026
Toe ash (%)	45.1	47.6	48.5	49.6	46.6	46.9	46.6	0.82	0.385	0.223	0.220
Toe ash (mg)	372	436	488	530	412	439	447	11.9	0.073	<0.001	0.014
Foot ash[2] (g)	1.16	1.39	1.58	1.71	1.29	1.39	1.42	0.04	0.103	<0.001	0.019

[1] BD=basal diet, MSP_a=anhydrous monosodium phosphate, DCP_a=anhydrous dibasic calcium phosphate. [2] Foot ash=sum of tarsometatarsus and toe ash.

Amounts of P and Ca contained in the bones are presented in Table 11. In Period 1, a significant interaction ($P < 0.001$) between the level and the source of P for both Ca and P contents in all bones was found. Both the level and the source of dietary P significantly ($P \leq 0.003$) affected the amounts of P and Ca in tibia, tarsometatarsus, and toe. The birds fed MSP_a had significantly higher amounts of Ca and P in their bones than the birds fed DCP_a. In Period 2, significant interactions ($P < 0.05$) between the level and the source of P were found for amounts of both Ca and P for all bones, except for tibia Ca (Table 11). The effect of the level of P was significant for all criteria ($P < 0.001$), while the source of P had no significant effect on the amounts of P and Ca in Period 2.

All tibia criteria measured by QCT were significantly affected ($P < 0.001$) by the P level in the diets in Period 1 (Table 12). Increasing the P level in the diet by either P source increased TD, CD, SSI, and CC. The P source had a significant effect on TD ($P = 0.037$), CC ($P = 0.017$), and SSI ($P = 0.014$), but not on CC. There was a significant interaction ($P \leq 0.001$) between the level and the source of P for TD, CC, and SSI. In Period 2, the P level in the diet had a significant effect ($P < 0.05$) on all QCT measurements and tibia breaking strength, while the P source had not. No significant interactions were found.

4.4.2. SERUM P_I AND CA

The blood serum P_i concentration was significantly affected ($P < 0.001$) by the P level in the diet but not by the P source or their interaction in Period 1 (Table 13). In Period 2, serum P_i also was significantly increased by the dietary P level and a significant interaction ($P < 0.001$) was detected, showing that the DCP_a supplementation increased serum P_i to a lesser extent than the MSP_a supplementation. The serum Ca concentration was neither affected by the P source nor the P level or their interaction in both periods.

Table 11. Amount of P and Ca (mg) determined in bones of broilers at different ages following feeding diets with different inclusion levels of two different mineral P sources

	BD[1]	MSP_a			DCP_a			Pooled	*P* (ANOVA)		
		0.08%	0.16%	0.24%	0.08%	0.16%	0.24%	SEM	P source (S)	P level (L)	LxS
Period 1											
Tibia P	50	61	84	102	61	72	82	1.69	<0.001	<0.001	<0.001
Tibia Ca	107	135	185	222	131	158	179	4.79	0.003	<0.001	<0.001
Tarsometatarsus P	28	36	50	61	34	41	47	0.99	<0.001	<0.001	<0.001
Tarsometatarsus Ca	56	74	103	127	69	84	96	2.11	<0.001	<0.001	<0.001
Toe P	12	16	22	28	15	18	21	0.55	<0.001	<0.001	<0.001
Toe Ca	26	34	47	61	32	39	45	1.21	<0.001	<0.001	<0.001
Period 2											
Tibia P	274	331	379	406	310	333	340	9.48	0.112	<0.001	0.021
Tibia Ca	574	697	789	831	648	697	708	23.2	0.321	<0.001	0.114
Tarsometatarsus P	141	170	198	215	158	171	177	5.19	0.081	<0.001	0.013
Tarsometatarsus Ca	298	364	420	454	336	365	377	11.8	0.166	<0.001	0.038
Toe P	77	92	105	115	86	94	95	3.36	0.116	<0.001	0.034
Toe Ca	156	184	204	221	173	188	187	6.00	0.148	<0.001	0.049

[1] BD=basal diet, MSP_a=anhydrous monosodium phosphate, DCP_a=anhydrous dibasic calcium phosphate.

Table 12. Results of QCT measurements and breaking strength for tibiae of broilers at different ages following feeding diets with different inclusion levels of two different mineral P sources

	BD[1]	MSP_a			DCP_a			Pooled	*P* (ANOVA)		
		0.08%	0.16%	0.24%	0.08%	0.16%	0.24%	SEM	P source (S)	P level (L)	LxS
Period 1											
Total density (mg/cm^3)	304	391	458	586	320	399	434	11.3	0.037	<0.001	0.001
Cortical density (mg/cm^3)	451	552	601	716	474	551	591	11.0	0.398	<0.001	0.051
Cortical content (mg/mm)	3.2	4.4	5.8	7.2	3.9	5.0	5.8	0.13	0.017	<0.001	<0.001
Strain Strength Index (mm^3)	2.4	4.6	7.5	10.6	3.5	5.8	7.6	0.27	0.014	<0.001	<0.001
Period 2											
Total density (mg/cm^3)	410	436	544	558	410	456	488	12.4	0.370	<0.001	0.109
Cortical density (mg/cm^3)	724	759	877	901	740	790	833	14.6	0.632	<0.001	0.276
Cortical content (mg/mm)	18.5	21.4	23.6	24.0	21.0	22.2	22.3	0.50	0.338	<0.001	0.187
Strain Strength Index (mm^3)	39.0	46.6	52.6	52.5	45.6	48.8	48.4	1.86	0.557	0.023	0.403
Breaking strength (N)	182	209	240	239	208	226	231	8.57	0.628	0.005	0.523

[1] BD=basal diet, MSP_a=anhydrous monosodium phosphate, DCP_a=anhydrous dibasic calcium phosphate.

Table 13. Concentration of P_i and Ca in blood serum of broilers at different ages following feeding diets with different inclusion levels of two different mineral P sources (mmol/L)

	BD[1]	MSP_a			DCP_a			Pooled	*P* (ANOVA)		
		0.08%	0.16%	0.24%	0.08%	0.16%	0.24%	SEM	P source (S)	P level (L)	LxS
Period 1											
Serum P_i	1.75	1.85	2.11	2.53	1.84	1.97	2.28	0.10	0.318	<0.001	0.224
Serum Ca	3.45	3.78	3.76	3.54	3.71	3.83	3.67	0.09	0.308	0.138	0.268
Period 2											
Serum P_i	1.46	1.62	2.17	2.68	1.58	1.66	1.85	0.07	<0.001	<0.001	<0.001
Serum Ca	2.86	2.71	2.43	2.58	2.80	2.89	2.86	0.08	0.514	0.660	0.244

[1] BD=basal diet, MSP_a=anhydrous monosodium phosphate, DCP_a=anhydrous dibasic calcium phosphate.

4.4.3. COMPARISON OF SLOPES

The relationship between the dietary P concentration and the different response criteria was linear (best fit for straight line in comparison with quadratic or cubic models) in Periods 1 and 2. All regressions are displayed in Table 14 (Period 1) and 15 (Period 2). Figure 3 shows this relationship for the amount of tarsometatarus ash for both periods as an example. In Period 1, slopes determined for DCP_a were significantly lower for tibia ash and P, tarsometatarsus ash and P, and amount of toe ash and P than for MSP_a (Table 14). They were also significantly lower for the QCT measurements. Differences between slopes were not significantly different for serum P_i and toe ash concentration. In Period 2, the comparison of slopes yielded similar results. But in contrast to Period 1, slopes between DCP_a and MSP_a were not significantly different for tibia cortical content and SSI (Table 15). They were, however, significant for serum P_i concentration. The ratio of the slopes was different between the response criteria. It varied between 1.90 and 1.42 in Period 1, and 3.31 and 1.94 in Period 2 (Table 16).

Table 14. Estimated parameters of linear regressions calculated for criteria of P evaluation depending on the dietary P concentration in Period 1

Criterion	Phosphate	Intercept	Slope	R^2	$S_{y.x}$	*P* (slopes)
Tibia ash, %	MSP_a	26.8	4.1	0.73	2.3	0.012
	DCP_a	33.5	2.3	0.40	2.6	
Tibia ash, g/bone	MSP_a	-0.18	0.13	0.94	0.03	<0.001
	DCP_a	0.01	0.08	0.89	0.03	
Tibia P, mg/bone	MSP_a	-32.7	22.7	0.94	5.1	<0.001
	DCP_a	1.1	13.9	0.89	4.4	
Tarsometatarsus ash, %	MSP_a	17.8	4.9	0.79	2.3	0.006
	DCP_a	24.7	3.0	0.54	2.5	
Tarsometatarsus ash, g/bone	MSP_a	-0.13	0.078	0.95	0.016	<0.001
	DCP_a	-0.001	0.045	0.86	0.016	
Tarsometatarsus P, mg/bone	MSP_a	-24.1	14.3	0.95	2.9	<0.001
	DCP_a	-1.2	8.2	0.89	2.6	
Toe ash, %	MSP_a	38.5	2.0	0.60	1.5	0.104
	DCP_a	41.5	1.2	0.28	1.8	
Toe ash, mg	MSP_a	-67.2	37.3	0.94	8.5	<0.001
	DCP_a	-8.5	21.5	0.86	7.8	
Toe P, mg	MSP_a	-12.8	6.8	0.94	1.6	<0.001
	DCP_a	-1.8	3.9	0.86	1.5	
Foot ash, g	MSP_a	-0.20	0.12	0.96	0.02	<0.001
	DCP_a	-0.014	0.07	0.88	0.02	
Total density, mg/cm^3	MSP_a	-104	114	0.89	37	<0.001
	DCP_a	84	60	0.77	30	
Cortical density, mg/cm^3	MSP_a	83	105	0.88	36	<0.001
	DCP_a	220	63	0.77	32	
Cortical content, mg/mm	MSP_a	-2.9	1.7	0.96	0.32	<0.001
	DCP_a	-0.9	1.2	0.87	0.41	
Strain Strength Index, mm^3	MSP_a	-10.1	3.5	0.95	0.72	<0.001
	DCP_a	-5.9	2.3	0.88	0.78	
Serum P_i, mmol/L	MSP_a	0.53	0.33	0.55	0.27	0.188
	DCP_a	0.94	0.22	0.30	0.30	
BWG, g	MSP_a	200	40.4	0.47	40	0.808
	DCP_a	213	37.9	0.48	36	

Table 15. Estimated parameters of linear regressions calculated for criteria of P evaluation depending on the dietary P concentration in Period 2

Criterion	Phosphate	Intercept	Slope	R^2	$S_{y.x}$	*P* (slopes)
Tibia ash, %	MSP_a	31.4	3.0	0.73	1.7	0.001
	DCP_a	36.5	1.5	0.50	1.4	
Tibia ash, g/bone	MSP_a	0.58	0.28	0.78	0.14	<0.001
	DCP_a	1.1	0.14	0.45	0.14	
Tibia P, mg/bone	MSP_a	86	55	0.80	26	<0.001
	DCP_a	185	28	0.47	27	
Tarsometatarsus ash, %	MSP_a	29.9	2.5	0.54	2.1	0.007
	DCP_a	35.6	0.82	0.11	2.1	
Tarsometatarsus ash, g/bone	MSP_a	0.23	0.16	0.78	0.08	<0.001
	DCP_a	0.52	0.081	0.46	0.08	
Tarsometatarsus P, mg/bone	MSP_a	32.9	31.4	0.82	13.7	<0.001
	DCP_a	90.2	15.2	0.47	14.7	
Toe ash, %	MSP_a	39.3	1.8	0.32	2.4	0.072
	DCP_a	43.6	0.6	0.05	2.3	
Toe ash, mg	MSP_a	0.15	0.065	0.77	0.03	<0.001
	DCP_a	0.27	0.032	0.45	0.03	
Toe P, mg	MSP_a	21.9	16	0.74	8.6	0.002
	DCP_a	51.5	7.8	0.37	9.4	
Foot ash, g	MSP_a	0.38	0.23	0.79	0.10	<0.001
	DCP_a	0.79	0.11	0.46	0.11	
Total density, mg/cm^3	MSP_a	159	70	0.71	42	0.004
	DCP_a	272	36	0.44	37	
Cortical density, mg/cm^3	MSP_a	428	82	0.72	47	0.009
	DCP_a	546	48	0.52	42	
Cortical content, mg/mm	MSP_a	10.8	2.4	0.69	1.4	0.070
	DCP_a	13.6	1.6	0.48	1.5	
Strain Strength Index, mm^3	MSP_a	20.3	5.8	0.50	5.3	0.211
	DCP_a	27.1	3.9	0.31	5.3	
Serum P_i, mmol/L	MSP_a	-0.51	0.53	0.80	0.24	<0.001
	DCP_a	0.89	0.16	0.44	0.17	
Breaking strength, N	MSP_a	99	25.2	0.48	24	0.499
	DCP_a	115	20.7	0.42	22	
BWG, g	MSP_a	675	41.4	0.22	76	0.124
	DCP_a	805	12.0	0.03	61	

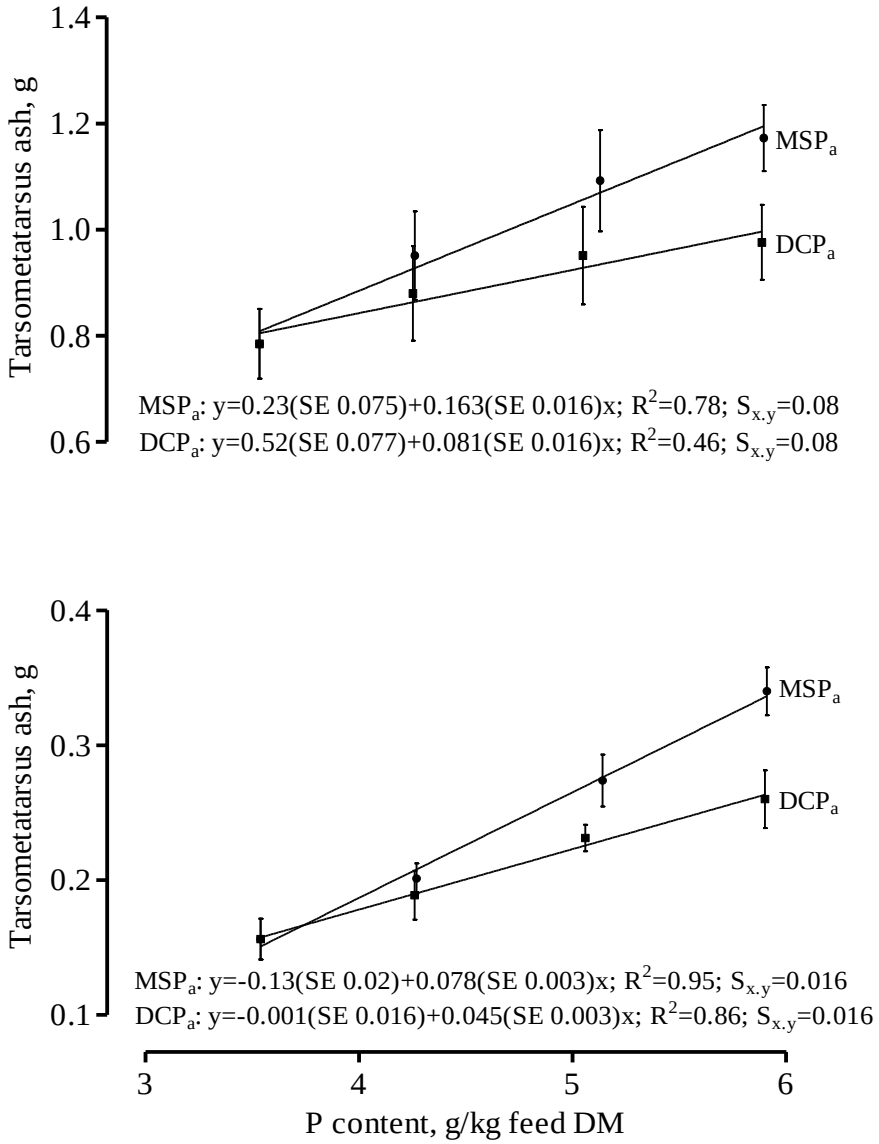

Figure 3. Tarsometatarsus ash depending on P concentration of the diet in Period 1 (lower panel) and 2 (upper panel) (mean and SD; n=8 replicates per treatment)

Table 16. Ranking[1] of the slope ratios for different response criteria

	Ranking	Criterion	Slope ratio (MSP_a:DCP_a)
Period 1		P retention, g/kg feed DM[2]	2.41
	1	Pc digestibility, g/kg feed DM	2.23
	2	Total density, mg/cm^3	1.90
	3	Tibia ash, %	1.80
	4	Toe P, mg	1.74
	5	Tarsometatarsus-P, mg/bone	1.74
	6	Toe ash, mg	1.74
	7	Tarsometatarsus ash, g/bone	1.73
	8	Foot ash, g	1.71
	9	Cortical density, mg/cm^3	1.67
	10	Tarsometatarsus ash, %	1.63
	11	Tibia P, mg/bone	1.63
	12	Tibia ash, g/bone	1.63
	13	Strain Strength Index, mm^3	1.52
	14	Cortical content, mg/mm	1.42
Period 2		P retention, g/kg feed DM	2.17
	1	Pc digestibility, g/kg feed DM	2.16
	2	Foot ash, g	2.09
	3	Tarsometatarsus P, mg/bone	2.07
	4	Toe P, mg	2.05
	5	Toe ash, mg	2.03
	6	Tibia ash, g/bone	2.00
	7	Tibia ash, %	2.00
	8	Tarsometatarsus ash, g/bone	1.98
	9	Tibia P, mg/bone	1.96
	10	Total density, mg/cm^3	1.94
	11	Tarsometatarsus ash, %	3.05
	12	Serum P_i, mmol/L	3.31

[1]Only for criteria with significant differences between the slopes for MSP_a and DCP_a.

[2]Data for P retention and digestibility were taken from Shastak et al. (submitted to Poultry Science).

4.5. DISCUSSION

4.5.1. BONE ASH AND P

Ash concentration of tibia is often used to estimate the degree of bone mineralization in broilers. However, the goodness of fit (R^2) in our experiment was much higher and the standard error lower for the amounts of ash in tibia, tarsometatarsus, and toe than for their respective concentrations. This is in agreement with Hall et al. (2003) who also found much higher R^2 for bone ash amount than for bone ash percentage (0.92 vs. 0.57). The authors suggested that the amount of tibia ash is the more sensitive indicator of bone mineralization. The ash concentration is in fact a ratio of two absolute values, and the absolute values seem to be more influenced by breed, age, and feed intake of the birds than the relative values (Huyghebaert et al., 1980). Coon et al. (2007) also reported that the slope of percentage tibia ash was not an accurate method for determining relative biological availability in their study compared with the slope of tibia ash amount.

The slope ratios (MSP_a:DCP_a) of the linear regression for the amounts of ash in tibia, tarsometatarsus, toe, and foot were very similar within Period 1 (1.63, 1.73, 1.74, and 1.71) and Period 2 (2.00, 1.98, 2.03, and 2.09). The R^2 between tibia ash amount vs. tarsometatarsus or toe ash amount in Period 1 was 0.97 and 0.94, respectively. R^2 for these criteria in Period 2 were only slightly lower than in Period 1 (0.94 and 0.89, respectively). Therefore, tarsometatarsus or toe ash could be used instead of tibia ash in estimating the availability of mineral phosphates, both leading to similar results. Toe ash also was reported to be as sensitive as tibia ash in assessing biological availability of P by Fritz and Roberts (1968) and Yoshida and Hoshii (1977, 1983). In contrast, Kornegay and Yi (1999) reported that the use of tibia ash as the response criterion resulted in a ranking of P availability values different from that obtained with toe ash.

There was a very good relationship between the amounts of tibia ash and foot ash in Period 1 and 2 (R^2 = 0.96 and 0.94, respectively). Yan et al. (2005) also showed a close relationship between fat extracted tibia ash (%) and foot ash (%) (R^2 = 0.92) or toe ash (%) (R^2 = 0.88) in 3-wk-old broilers. Fat extraction did not affect the sensitivity of the assays, and both defatted and undefatted bones lead to similar results (Yan et al., 2005; Garcia and Dale, 2006). Garcia and Dale (2006) used foot ash for quantifying bone mineralization. They assumed that foot ash might represent the bone mineralization status more accurately than toe ash because of the greater number of bones included in the determination. We obtained the data using bones cleaned from the soft and connective tissues, which probably yielded more

accurate results than those obtained by Yan et al. (2005) and Garcia and Dale (2006). Thus, we conclude from our data and in agreement with Dale and Garcia (2006) that foot ash is as sensitive as tibia ash. This is relevant because it is easier to obtain a foot than a tibia bone for P availability estimates. Additionally, it is not always clear from published toe ash studies which bones were taken (all toes, middle, or another toe), at which phalanxes were they cut, and whether this affected the results. This shortcoming can be avoided when foot ash obtained from the whole foot and cut at the tibiotarsometatarsal joint is used.

The slope ratios calculated for MSP_a and DCP_a using amounts of P contained in tibia, tarsometatarsus, and toe were almost identical with ratios calculated for bone ash amounts in Period 1 (Table 7). The comparison of amounts of P and ash for tibia, tarsometatarsus, and toe bones revealed an R^2 of 0.99 for all three bones. It can be concluded that using bone ash amount alone is as precise as the amount of P for the ranking of P availability of different P sources in 3-wk-old broilers and that the additional analysis of P is not needed for this purpose.

The P concentration in bone ash was not affected by the level or source of P in the diet. For instance, in Period 1 (Period 2) there were 169 (179), 166 (180), 169 (182), 170 (184), 170 (179), 170 (179), and 170 (181) g P/kg tibia ash for the BD, 0.08%, 0.16%, and 0.24% MSP_a or DCP_a supplemented diets, respectively. Ca percentage in bone ash varied little among species or anatomical location of bones (Field et al., 1974), what is also valid for P because of their relationship as the components of hydroxylapatite (Ca:P = 2:1) (Field, 1999). Ash concentration in bones may be decreased by low Ca or P diets but the Ca concentration in ash remains constant at approximately 37% (Field, 1999), which is in agreement with our data (37% and 38% Ca in ash for tibia and tarsometatarsus in Periods 1 and 2, respectively; 39% and 42% Ca in ash for toes in Period 1 and 2, respectively). P (Ca) contents in g per kg ash were 179 (368) and 180 (385) in Period 1 and 180 (383) and 212 (421) in Period 2 for tarsometatarsus and toe bones, respectively. However, variability in Ca or P in bone ash might be related to the amount of marrow in bone, cartilage attached to the bone, or lean, fat, and tendon on the surface of the bone when it is ashed (Field, 1999). Variation was low in our study possibly due to the way we have cleaned the bones.

4.5.2. QCT MEASUREMENTS

TD, CD, CC, and SSI measured by QCT showed similar trends compared to the amount of tibia ash and tibia P. In Period 1, TD and CD of tibiae had a good relationship with the tibia ash amount (R^2 = 0.78 and 0.74, respectively). A close relationship (R^2 = 0.78) between TD

determined by QCT and tibia ash amount was not unexpected due to the fact that the bone density is determined by the amount of hydroxylapatite. Hydroxylapatite and other minerals comprise approximately 70% of the dry, fat-free mature bone weight (Field, 1999). Therefore, the more P is available, the more bone tissue can be formed.

The highest R^2 were obtained between tibia ash amount and CC (0.94) in Period 1. If the supply of Ca and P in the diet is inadequate, both minerals can be obtained by increased bone resorption or by reduced bone formation within the osteons of the cortical bone (Williams et al., 2000). This makes the cortical a very sensitive area of the bone (Williams et al., 2000). As the indicator of the stability of the bone towards bending or torsion, SSI also showed a very good relationship to the amount of tibia ash (R^2 = 0.93) in Period 1.

In Period 2, QCT measurements had a poor relationship with the tibia ash amount (R^2 = 0.39 and 0.31 for TMD and CD, respectively). An explanation may be the higher fat content of bones in 5-wk-old birds. Data from Edwards et al. (1973) showed rapid linear increase in body fat concentration with increasing age of broilers. The level of fat in the tibiotarsus of 40-d-old broilers was about 18±10 g/kg bone DM (Suchy et al., 2009). However, fat is mainly localized in the bone marrow (Field, 1999). The biggest source of error in the single-energy X-ray QCT systems is the fat within the bone marrow (Taicher et al., 2003), which might impact the results. A slightly excessive P intake in the highest P supplementation level in relation to the requirement for bone mineralization might also have been a reason for the poor relationship. However, the accuracy of QCT may be improved by performing scans at two different X-ray energy levels (dual-energy QCT) (Taicher et al., 2003). Onyango et al. (2003) using DEXA showed that bone densitometry criteria (bone mineral content and bone mineral density) were highly correlated with bone ash, which indicated that bone densitometry is an efficient method to measure bone mineralization in broilers. However, by using the DEXA method only areal determination of mineral density (g/cm^2) is possible. QCT allows measuring the true volumetric bone density in the sense of mass per unit volume (g/cm^3). Korver et al. (2004) concluded that the technology was suitable to follow changes in bone mineral density in meat-type birds as they grow and remodel their skeletal structures to support increased body mass. The peripheral QCT used in our experiment allows measurements only in a defined section of a long bone, which might make this method less sensitive. While the fact that the entire bone is not measured is not necessarily a disadvantage, it must be kept in mind when interpreting QCT results (Korver et al., 2004). Our data showed that the evaluation of the defined bone sector (50% of the tibia length) may provide almost as good results as the whole tibia ash amount in Period 1. Thus, QCT measurements, especially

CC and SSI, can be used to predict bone mineralization or its mechanical properties at this age.

4.5.3. PERFORMANCE, BLOOD AND BONE BREAKING STRENGTH

In Period 2, there was no significant difference ($P > 0.05$) between the slopes (MSP_a vs. DCP_a) for tibia breaking strength, which indicates that it is not a sensitive indicator. Poor sensitivity of tibia breaking strength or shear force was also reported by Huyghebaert et al. (1980), Ravindran et al. (1995), and Kornegay and Yi (1999). However, values for tibia breaking strength may be affected by the type of instrument used, procedures used to prepare the bones for testing, and physical and mechanical properties of bones (Orban et al., 1993).

Serum P_i with a slope ratio of 1.5 was not sensitive enough ($P > 0.05$ between the slopes of linear regressions) to detect difference between the two phosphates sources evaluated in Period 1. In Period 2, the difference between the slopes for serum P_i was significant ($P < 0.001$); nevertheless, the slope ratio of 3.27 was much greater than that determined for tibia ash or P retention (Table 8). The levels of P_i and Ca in blood are regulated by parathyroid hormone (PTH), thyrocalcitonin (TCT), and calcitriol (CT). When plasma Ca^{2+} and /or P_i are too low, PTH followed by CT are released to increase plasma Ca^{2+} and/or P_i by intestinal absorption and bone resorption and to reduce Ca and P excretion by the kidney (Veum, 2010). Conversely, when plasma Ca^{2+} and /or P_i are too high, TCT reduces the intestinal absorption and the bone resorption of Ca and P and increases excretion by the kidney (Veum, 2010). This complex regulation lets plasma concentrations appear of low value in P availability studies. This is in agreement with Gueguen (1999) and De Groote and Lippens (2002) who considered alkaline phosphatase and serum or plasma P not to be suitable for determining P bioavailability in poultry.

BW gain and feed per gain ratio had some of the lowest R^2 from the criteria evaluated in Period 1 and 2 (Tables 6 and 7). There was neither any significant difference ($P > 0.05$) between the slopes (MSP_a vs. DCP_a) in both periods. In confirmation of Huyghebaert et al. (1980) and Grimbergen et al. (1985), it is concluded that BWG and feed conversion cannot be used as indicators of P availability.

4.5.4. RANKING OF RESPONSE CRITERIA EVALUATED RELATIVE TO P RETENTION

The studies described here were linked with another study that determined and compared the availability of the mineral phosphates based on P retention and prececal (**pc**) digestibility (Shastak et al., submitted to Poultry Science). Relative availability values based on bone ash

or blood P_i caused a different ranking for mineral P sources in comparison with retention data (Table 8). Edwards and Gillis (1959) used a balance approach for evaluating P availability in Leghorn cockerels fed a purified diet (0.03 to 0.05% P unsupplemented). Consistency was found to be only reasonable between the balance approach and bone ash regression method for evaluation of P sources. In the present study, none of the bone criteria or serum P_i in its ranking was close enough to P retention in Period 1. In Period 2, the differences between P retention and bone measures were fewer than in Period 1. However, bone ash responses may be affected by the duration of the experiment. Ammerman et al. (1961) reported that a 10-d bioassay was less sensitive than a 28-d bioassay for generating data for a tibia ash response curve. Nevertheless, the relative rank order of P availability from MSP_a and DCP_a remained the same (higher for MSP_a) either for P retention and pc digestibility or for all bone criteria. The consistency for both periods was the same only for P retention and pc digestibility and not for the bone criteria.

De Groote and Lippens (2002), based on a literature overview, gave the pc digestibility the highest priority for assessing the biological value of P sources in poultry. It was followed by P retention and bone ash and P (tibia/toe/metatarsal). The lowest ranking was given the tibia breaking strength and growth. This is in agreement with our data, if the pc digestibility would have been given the highest ranking (Table 8).

We conclude that foot ash amount might be used instead of tibia, tarsometatarsus, or toe ash in assessing bone mineralization in 3- as well in 5-wk-old broilers with the advantage of easier sampling if the skin, the soft, and the connective tissues removal is not necessary. QCT measurements can be used for assessing bone mineralization until the third week of age. The ranking of mineral P sources based on bone criteria differs from the ranking based on P retention or pc digestibility. This underlines the need for agreeing on a standard protocol for available P determination in poultry.

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CHAPTER 5

WHOLE BODY PHOSPHORUS TO TIBIA PHOSPHORUS RATIO IN BROILERS

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5. WHOLE BODY PHOSPHORUS TO TIBIA PHOSPHORUS RATIO IN BROILERS

5.1. INTRODUCTION

Maintaining phosphorus (P) resources in face of finite global rock phosphate stores has been identified to be one of the greatest challenges for sustainable food production (GROSS, 2010; NESET and CORDELL, 2011). This implies special challenges for the livestock industries (RODEHUTSCORD, 2008). In poultry production, precise knowledge about the birds' P retention and its variation is of high nutritional relevance for two reasons: First, because it is the main factor determining the P requirement; and second, because P availability of raw materials used in poultry feeding is often evaluated on the basis of P retention (RODEHUTSCORD, 2009).

Retention can be determined either from the difference between intake and excretion in balance studies or by comparative whole body analysis. Both approaches are laborious and expensive, and whole body analysis is an additional challenge in regard to obtaining a representative sample (HAAG, 1939). Hence, different bone characteristics, growth, or blood metabolites are used as an alternative to compare and rank different P sources regarding their availability (RODEHUTSCORD, 2009). Such relative availability data always depend on the response determined for a certain reference P source; therefore, the choice of the reference source affects the results. Moreover, the nutritionist today needs an actual retention value for key minerals to assess the true impact of dietary formulations on animal performance and on the elements remaining in animal excreta (COON et al., 2002). Determination of P retention, therefore, remains important.

With increases in prices for mineral P sources, the poultry and feed industries became increasingly interested in detailed information about the variation in availability of P between different raw materials, and even between different batches of the same raw material. The conflict between demand for data and effort for retention studies points to the question whether the retention of P in an individual bone can be considered as a reliable indicator of whole body P (WBP) retention. An early attempt to answer this question was made by HURWITZ (1964). He used the *tibiotarsus* (tibia) because it is easy to isolate and analyze. The mean ratio of WBP to tibia P (TP) in broilers was 19.6, but this value was based only on a low number of observations and did not refer to retention (HURWITZ, 1964). Since then, a

substantial improvement has been achieved in broiler performance, which is mainly associated with genetic changes (HAVENSTEIN et al., 2003). It is generally recognized that genetic selection for muscle growth in broilers has resulted in an imbalance between the developments of various body systems, including increased demands being placed on skeletal integrity (WILLIAMS et al., 2000). Thus, the modern broiler may differ from broilers almost 50 years ago, including the WBP to TP ratio.

The objective of this study was to investigate whether TP retention can be used to estimate WBP retention in a currently used broiler strain. The study included two different ages of birds. Variation in P retention of birds was caused by the level and the sources of P in the diet in order to achieve a wide range in P retention.

5.2. MATERIALS AND METHODS

The study was linked to digestibility studies that evaluated two different mineral P sources with broilers of different ages based on the regression approach (SHASTAK et al., submitted). Birds of the present study were placed in the same pens and received the same feed. For this reason, this paper includes only a brief description of feeding and management.

5.2.1. ANIMALS, MANAGEMENT AND DIETS

Unsexed broiler hatchlings (Ross 308) were obtained from Brüterei Süd (Regenstauf, Germany). Before the experiment started, birds were provided free access to a broiler starter diet containing (per kg) 238 g CP, 12.5 MJ ME, 10.6 g of Ca, and 7.1 g of P (4.5 g non-phytin P). In the experiment, a low-P corn-soybean meal-based basal diet was used (0.35% total P on dry matter (DM) basis) (Table 1). Anhydrous dibasic calcium phosphate (DCP_a; Sigma-Aldrich, Inc., Mo, USA) and anhydrous monosodium phosphate (MSP_a; Dr. Paul Lohmann GmbH KG, Emmerthal, Germany) were used to increment the P concentration in six further diets by 0.08%, 0.16%, and 0.24%. All diets were calculated to be below the P requirement of the broilers. The intended Ca:P ratio (both on total basis) was 2:1 in all diets. Diets were pelleted without using steam through a 3-mm die. Intended concentrations of P and Ca in the diets were confirmed by analysis (SHASTAK et al., submitted).

On day 11 and 25 of age, 560 chicks each were weighed individually and distributed among 56 floor pens with ten birds per pen. Each pen was randomly assigned to one of the seven diets with eight replicated pens per diet. Feed and water were available for *ad libitum* intake. After 10 days of feeding the experimental diets, all birds were asphyxiated with CO_2 for determination of precaecal digestibility (SHASTAK et al., submitted). In both age groups,

two animals from two different pens per treatment were selected randomly for whole body and tibia studies (14 individuals per age, equally distributed across diets). Another two birds were taken from the flock at the beginning of the experiment for baseline measurements.

Table 17. Ingredient composition of the basal diet and analyzed concentrations

Ingredient composition (g/kg)	
Corn	522
Soybean meal, solvent extracted, 51 % CP	190
Potato protein, 75 % CP	129
Corn starch	85
Soybean oil	20
D,L-methionine	1.5
Mineral mix[1]	1.0
NaCl	1.0
Sodium bicarbonate	3.0
Vitamin mix[2]	1.5
Choline chloride	2.0
Titanium dioxide	5.0
Exchange mixture[3]	39.0
Analyzed concentrations (g/kg dry matter)	
Crude protein	255
Crude fat	60
Calcium	7.1
Phosphorus	3.5

[1] Originally presented by SHASTAK et al. (submitted).

[2] Mineral mix (Celita SG 1,GFT MBH, Memmingen, Germany) provided per kg of diet: Cu, 15 mg; I, 1.6 mg; Fe, 90 mg; Mn, 120 mg; Zn, 80 mg; Co, 0.6 mg; Se, 0.5 mg.

[3] Vitamin mix (Raiffeisen Kraftfutterwerke Süd GmbH, Würzburg, Germany) provided per kg of diet: vitamin A, 9.000 IU; vitamin D_3, 2.250 IU; vitamin E, 22.5 mg; menadione, 1.8 mg; thiamine, 2.3 mg; riboflavin, 4.5 mg; niacin, 37.5 mg; Ca-D-Pantothenate, 10.5 mg; pyridoxine, 4.5 mg; vitamin B_{12}, 23 µg; folic acid, 0.75 mg; biotin, 0.075 mg.

[4] Exchange mixture contained cellulose and sand (1:1) and limestone. In six further diets this mixture was partially replaced by MSP_a or DCP_a.

5.2.2. WHOLE BODY AND TIBIA PREPARATION

The entire digestive tract was exposed immediately after the birds had been asphyxiated and weighed. Contents of crop, proventriculus, gizzard, intestine, and caecae were removed by flushing with water. Bodies including emptied organs were weighed again. Mass loss due to emptying the digestive tract accounted for 3.9 to 5.8% of the body weight of 21-day-old and 0.9 to 4.7% of 35-day-old broilers. "Whole body" in the context of this paper is defined as the body without the digestive tract content (digesta) but including both tibiae.

Both tibiae of each animal were removed, cleaned, and weighed. The tibiae-free body and the tibiae were frozen individually at -20°C. Later, the tibiae-free bodies including feathers and viscera were cut into small pieces and charred at 480°C for 8 h to reduce the formation of fume during subsequent incineration. Incineration was done in a muffle furnace (Nabertherm L 40/11, Nabertherm GmbH, Bremen, Germany) at 700°C for 24 h. Tibiae were dried for 24 h at 105°C and weighed again. Tibiae were separately ashed in a muffle furnace at 600°C for 24 h, and ashes were cooled in a desiccator and weighed.

5.2.3. CHEMICAL ANALYSES

Duplicate samples of the tibiae-free body ash were weighed (0.5 g) in weighing boats, transferred into glass tubes, and doused with 25 mL of nitric acid (65% (w/v), product no. 865.2500, for analysis, Th. Geyer GmbH & Co. KG, Renningen, Germany). The entire ash of each tibia was transferred into a glass tube and 25 mL nitric acid were added. Samples of the tibiae-free body ash and tibia ash were subsequently heated at 100°C for 40 min on a hotplate (block digestion system Behr K 20 L, Behr Labor Technik GmbH, Düsseldorf, Germany). After cooling, 50 mL of double distilled water were added to the solutions in the glass tubes. After mixing, the solutions were transferred into 500 mL Erlenmeyer flasks and filled up with double distilled water. Concentrations of Ca and P were measured at specific wavelengths for each element (Ca, 317.933; P, 213.618) by using an inductively coupled plasma emission spectrometer (VISTA PRO, Varian Inc., Australia).

5.2.4. CALCULATIONS

The amount of WBP and whole body Ca was calculated for each bird as the sum of the respective amounts determined in the tibiae-free body and the tibiae. Linear regressions were calculated using all data within one age in order to investigate relationships between TP and WBP. Regressions were calculated using GraphPad Prism 5.0 (Graph Pad Software, Inc.). In designing the study, we decided to use several levels of P with two replicated birds per

treatment instead of more replicates at the expense of P levels. Hence, a statistical comparison of treatment means was not appropriate.

5.3. RESULTS AND DISCUSSION

Whole body (WB) weight, P and Ca concentration in WB, P content in tibiae, and WBP/TP ratios for each bird are presented in tables 18, 19 and 20.

Table 18. Whole body weight (WBW), P and Ca concentration, P content in tibia, and whole body P (WBP)/tibia P (TP) ratio of broilers fed the starter diet until 10 days of age

Bird	Ration	WBW, g	P, g/kg WBW	Ca, g/kg WBW	P content[1] in tibia, mg	WBP/TP ratio
1	Starter	189	3.9	4.9	37.5	19.5
2	Starter	222	3.4	4.2	36.2	20.9

[1] Mean value of the left and right tibia.

Table 19. Whole body weight (WBW), P and Ca concentration, P content in tibia and whole body P (WBP)/tibia P (TP) ratio of broilers fed the experimental diets until day 21 of age

Bird	Ration	WBW, g	P, g/kg WBW	Ca, g/kg WBW	P content[1] in tibia, mg	WBP/TP ratio
1	Basal diet	672	2.3	2.5	67.8	22.9
2	MSP_a (0.08 % P)	679	2.5	2.7	72.7	22.9
3	MSP_a (0.16 % P)	794	3.6	4.8	132.4	21.6
4	MSP_a (0.24 % P)	695	3.9	5.5	133.3	20.5
5	DCP_a (0.08 % P)	691	2.4	2.6	73.3	23.0
6	DCP_a (0.16 % P)	734	2.7	3.4	93.2	20.9
7	DCP_a (0.24 % P)	607	3.4	4.4	103.8	20.0
8	Basal diet	707	2.2	2.4	69.7	21.9
9	MSP_a (0.08 % P)	668	2.7	3.1	86.0	21.0
10	MSP_a (0.16 % P)	727	3.4	4.4	129.2	19.2
11	MSP_a (0.24 % P)	774	3.7	4.8	143.0	20.0
12	DCP_a (0.08 % P)	695	2.9	3.3	88.2	22.9
13	DCP_a (0.16 % P)	700	3.0	3.2	102.8	20.4
14	DCP_a (0.24 % P)	684	3.0	3.4	94.9	21.3
	Mean (±SD)					21.3 ± 1.3

[1] Mean value of the left and right tibia.

Table 20. Whole body weight (WBW), P and Ca concentration, P content in tibia and whole body P (WBP)/tibia P (TP) ratios of broiler chickens fed the experimental diets until day 35 of age

Bird	Ration	WBW, g	P, g/kg WBW	Ca, g/kg WBW	P content[1] in tibia, mg	WBP/TP ratio
1	Basal diet	1709	3.1	4.1	248.0	21.0
2	MSP_a (0.08 % P)	2181	3.2	4.6	391.2	18.0
3	MSP_a (0.16 % P)	2357	4.1	5.5	469.0	20.5
4	MSP_a (0.24 % P)	1875	4.5	6.8	410.9	20.7
5	DCP_a (0.08 % P)	2442	3.0	3.4	367.5	20.0
6	DCP_a (0.16 % P)	1810	3.2	4.1	272.8	21.5
7	DCP_a (0.24 % P)	2340	3.6	4.7	441.8	19.2
8	Basal diet	1754	3.0	4.0	272.4	19.4
9	MSP_a (0.08 % P)	2035	3.6	5.2	400.9	18.5
10	MSP_a (0.16 % P)	2285	3.7	5.3	397.8	21.1
11	MSP_a (0.24 % P)	2269	4.1	5.6	505.3	18.5
12	DCP_a (0.08 % P)	2263	3.5	4.6	397.1	19.8
13	DCP_a (0.16 % P)	1860	4.1	5.6	402.6	18.8
14	DCP_a (0.24 % P)	2276	3.6	4.6	414.8	19.8
	Mean value					19.8 ± 1.1

[1] Mean value of the left and right tibia.

WB weight ranged between 672 and 774 g in 21-day-old and between 1709 and 2357 g in 35-day-old broilers. The WBP/TP ratio of the two 10-day-old birds was 19.5 and 20.9, respectively (Table 18). In 21-day-old birds, the P concentration in WB ranged from 2.2 to 3.9 g/kg (Table 19), and in 35-day-old birds from 3.0 to 4.5 g/kg (Table 20). At both ages, the P concentration in WB increased linearly and significantly with increasing P concentration in the diet (Figure 4). This was as expected because the supply of P was calculated to be below the requirement in all treatments. The difference between the slopes for MSP_a and DCP_a was significant ($P = 0.01$) in 21-day-old, but not significant in 35-day-old birds ($P = 0.09$).

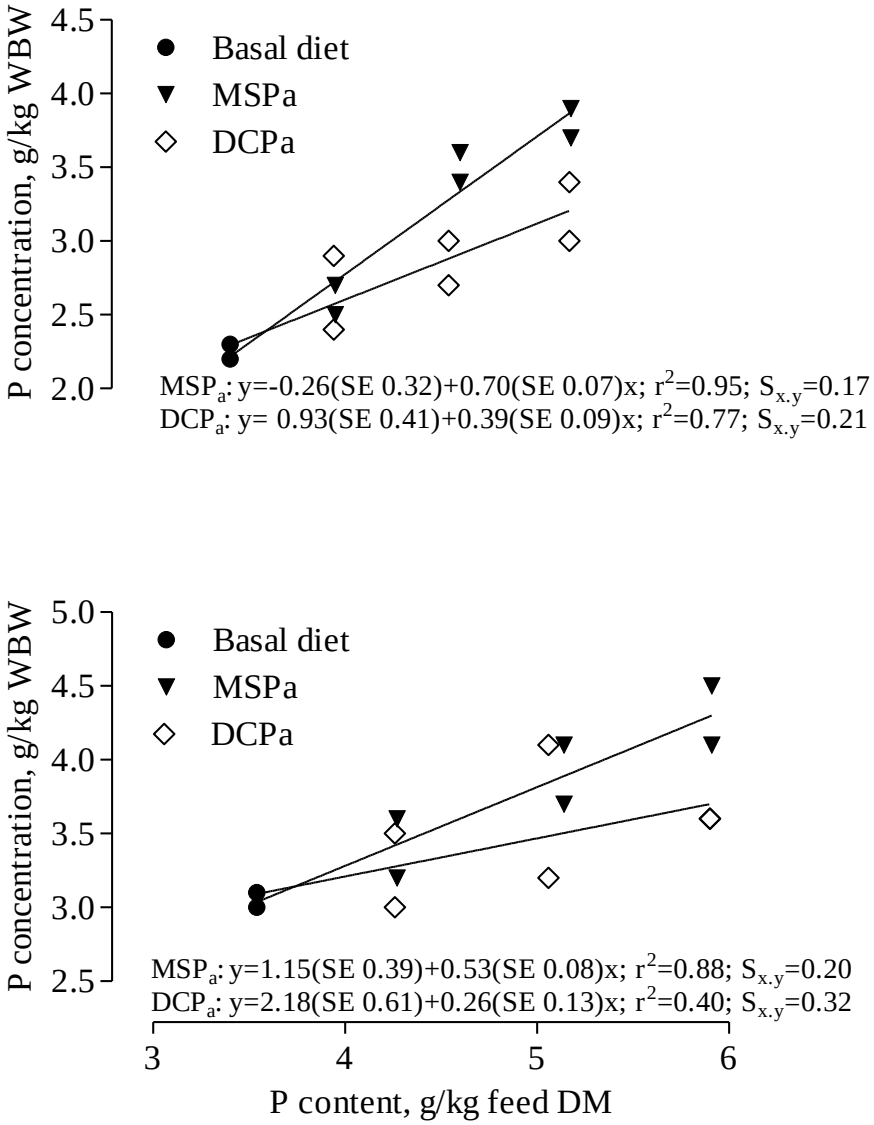

Figure 4. P concentration in the whole body (WB) depending on P concentration of the diet in 21-day-old (upper panel) and 35-d-old broilers (lower panel) (each data point represents one bird).

The amount of WBP as well as the tibia P content increased with the age of the broilers. The WBP/TP ratio in 21-day-old broilers was 21.3±1.3 (mean±SD, Table 3) while it was 19.8±1.1 in 35-day-old birds (Table 20). The linear regression between P content in the diet (g/kg DM) and WBP/TP ratio showed a negative slope that was calculated to be significantly different from zero for both P sources in 21-day-old birds *(P* = 0.04 and 0.03, respectively). Thus, the WBP/TP ratio at this age seems to be affected when P is supplemented to a diet that is very deficient in P. However, this observation was not made in 35-day-old birds, as slopes were calculated to be not significant from zero (*P* > 0.05).

HURWITZ (1964) used 16 chicks from 3 trials; the chicks were fed different P levels and analyzed for both carcass P and TP. The ratio between carcass P and TP was 19.6±1.0 at 24 days of age. This ratio was unaffected by the nutritional treatments used in his study. He concluded that the TP might be a good estimate of carcass P. HUYGHEBAERT et al. (1980) determined a body P/TP ratio of 17.95±0.5 in 28-day-old broilers fed diets with less than 0.6% P. With further increase in P above this level the ratio declined to 16.8±0.8. The researchers supposed the P level of the tibia to be a good indicator for the amount of total P retained, when the percentage of P in the diet was less than 0.6%. However, they used only one inorganic P source to establish this ratio. Neither HURWITZ (1964) nor HUYGHEBAERT et al. (1980) made any statistical analyses to investigate whether the WBP/TP ratio was affected by dietary treatments used in their studies.

Different WBP/TP ratios were determined in different studies: 19.6 (HURWITZ, 1964) vs. 17.95 or 16.8 (HUYGHEBAERT et al., 1980) vs. 21.3 or 19.8 in the present study. This may have had different reasons. First, the length of the experimental period was different between studies. We used a ten-day trial, whereas HURWITZ (1964) and HUYGHEBAERT et al. (1980) used 16 days and 21 days, respectively. There might be a discrepancy in mineralization of different bones in the bird's body (KORNEGAY and YI, 1999). The zone of proliferation in the developing bone of the young chick is especially sensitive to nutritional deficiencies and is quickly influenced by a P deficiency (NELSON and WALKER, 1964). The tibia bone is one of the most sensitive bones to P deprivation (McLEAN and URIST, 1961). Thus, the difference between WBP/TP ratios might be affected by the length of the P deprivation. Second, at 21 days of age the WBP/TP ratio in our study tended to be greater than at 35 days of age (21.3 vs. 19.8). This could be explained by different growth rates of various bones in the chicken's body. The tibia shows the highest rate of growth in length of all long bones in the chick body (CHURCH and JOHNSON, 1964). However, this growth rate changes depending on the bird's age, which could have led to differences in the WBP/TP ratio between the two age periods and even between studies. Moreover, this might at least partially explain that the relationship between the P content in the diet (g/kg DM) and the WBP/TP ratio was significantly affected ($P < 0.05$) in 21-day-old but not in 35-day-old broilers. Because tibiae are more affected by P deficiency than other bones in the chicken's body, this possibly also leads to a wider WBP/TP ratio in younger birds. And third, the genetics and productivity of birds have changed dramatically in comparison to the chicks used by HURWITZ (1964) and HUYGHEBAERT et al. (1980), which might have led to an alteration of this ratio in modern-type broilers. WILLIAMS et al. (2000) compared the bone

development of fast-growing meat-type chickens and slow-growing chickens of the same broiler line that had not been selected for growth performance since 1972, under typical commercial conditions. The authors found that the ash content in different sections of the tibia was consistently lower and the bone more porous in the birds selected for high performance. Moreover, the ratios of 21.3 in 21-day-old and 19.8 in 35-day-old broilers only represent the WBP/TP at the given of age. As our experiment and the studies cited above have shown, there might be a variation in this value between studies and experimental periods. Thus, the WBP/TP ratio seems to be not as constant as assumed by HURWITZ (1964), at least not with respect to the bird's age and the P level in the diet or both.

Changes in WBP and TP reflect the increments in retention due to increased P intake. The increase in WBP, when related to the increase in TP, was linear and the slope 17.7 at both ages (Figure 5). Hence, with each mg of P retained in the tibia the whole body P retention increased by 17.7 mg. This might make it possible to calculate a quantitative P retention from a given feed phosphate based only on the TP retention. To do this, the amount of TP at the beginning of an experiment is needed and representative birds need to be sampled for baseline measurements. Then P intake from the basal diet and from the P supplement has to be studied before the tibia is sampled from the experimental birds. This might be an alternative to performing a balance trial with excreta collection for the evaluation of P supplements.

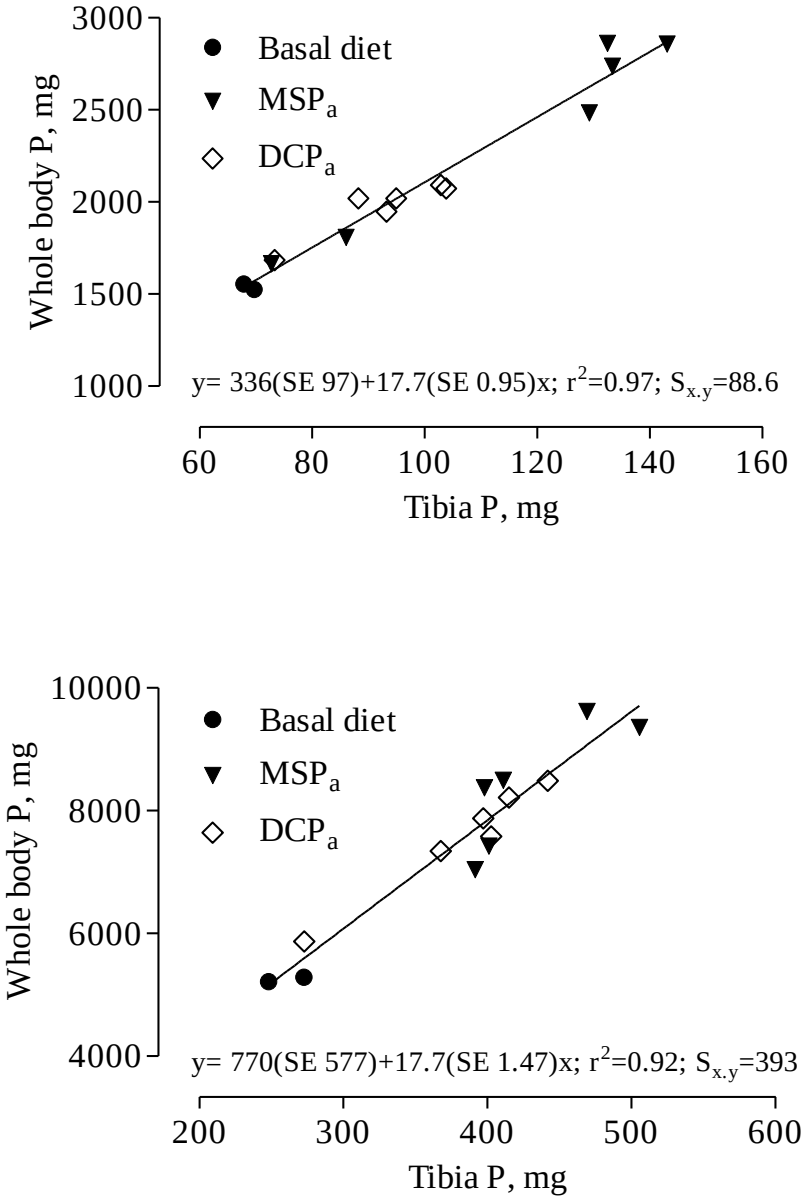

Figure 5. The linear relationship between tibia P and whole body P examined in 21-day-old (upper panel) and 35-day-old broilers (lower panel).

The Ca concentration per kg WB ranged from 2.4 to 5.5 g in 21-day-old and from 3.4 to 6.8 in 35-day-old birds. The Ca:P ratio in the whole body was about 1.3 in 10-day-old birds (Table 18). Nearly the same value (1.3) was reported by WPSA (1985) for newly hatched chicks. The Ca:P ratio in the whole body for three- and six-week-old broilers was given to be 1.4:1 at sufficient P and Ca supply (WPSA, 1985). This is in agreement with our data for the 35-day-old birds. In this age period, we also obtained an average Ca:P ratio of 1.4:1. The Ca:P ratio in 21-day-old birds was 1.2:1 (Table 19). This lower value can be explained by the

suboptimal Ca and P supply in the earlier phase of bone growth. 80% to 85% of the total body P (DRIVER, 2004) and about 99% of the total body Ca (HURWITZ et al., 1987) is present in the bones. The periods of rapid bone formation and mineralization are ended in broilers by the third week of age (WILLIAMS et al., 2000). Numerous substitutions and deletions in the crystal lattice maintain chemical reactivity, allowing bone to fulfill its role as a mineral reservoir; this can also alter the molar Ca:P ratio of the mineral (WILLIAMS et al., 2000). However, the linear relationship between whole body Ca and WBP in both age periods was very similar (Figure 6), meaning that with each retained gram of Ca the WBP content increased by about 0.6 g.

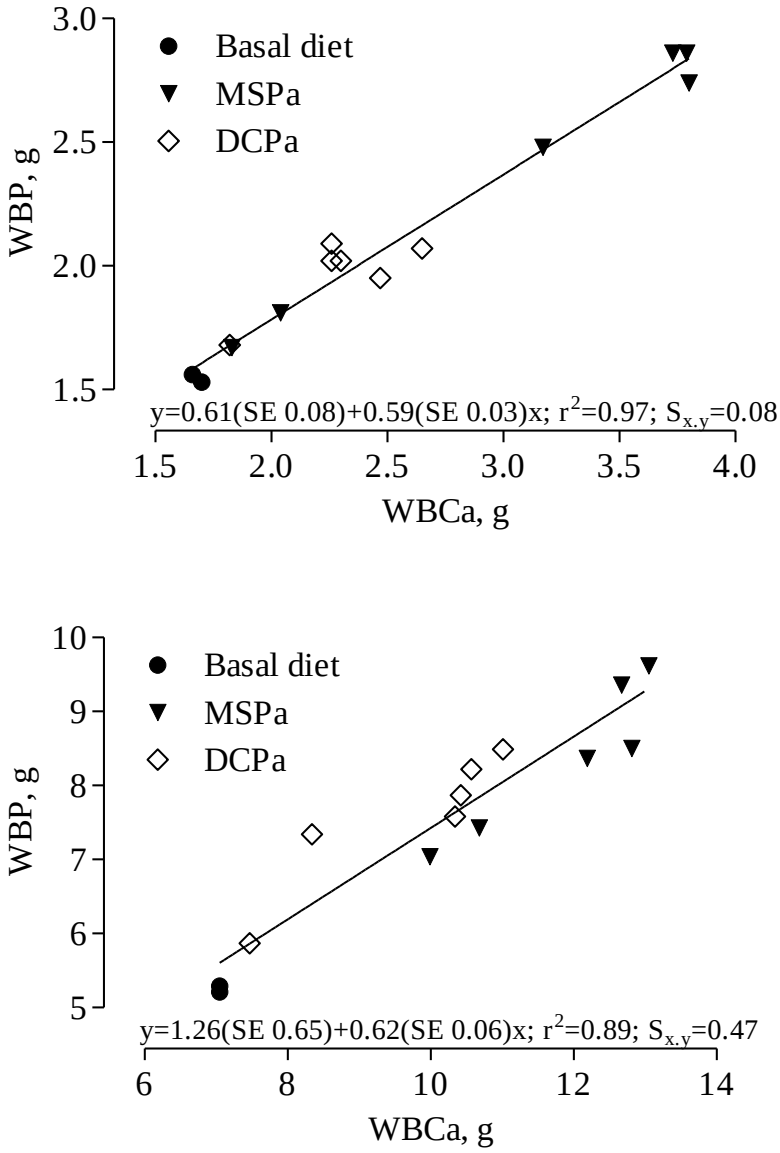

Figure 6. The linear relationship between whole body Ca (WBCa) and whole body P (WBP) examined in 21-day-old (upper panel) and 35-day-old broilers (lower panel).

We conclude that TP retention may be a suitable criterion to determine WBP retention. Differences between broiler strains or avian species need further investigation before it can be evaluated whether the ratio of WBP retention and TP retention that we found (17.7) can be applied in general.

5.4. AKNOWLEDGEMENT

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5.5. SUMMARY

The objective of this study was to investigate whether tibia phosphorus (TP) retention can be used to estimate whole body phosphorus (WBP) retention in broilers. A corn-soybean meal-based basal diet was used (0.31% total P). Anhydrous monosodium phosphate or anhydrous dibasic calcium phosphate was supplemented to increment the total P content by 0.08%, 0.16%, and 0.24%. The ratio of total Ca to total P was 2:1 in all diets. Mixed-sex Ross 308 broilers were raised in floor pens. On days 11 and 25 of age, birds were allocated to one of the diets, and feed was offered for *ad libitum* intake. On days 21 and 35, animals were asphyxiated, two birds per treatment were randomly chosen, and the remaining birds were kept for another study. Tibiae were isolated and the digestive tract content removed. The whole bodies including feathers and viscera were cut into pieces, incinerated, and ashes were collected. Tibiae were ashed separately. Contents of P and Ca in both fractions were quantified. At day 21, the WBP/TP ratio was 21.3±1.3 (mean and SD). At 35 days, the ratio was 19.8±1.1. The slope of linear regressions between the TP and the WBP for both ages was identical (17.7). Results indicated that changes in tibia P may be suitable to predict changes in whole body P retention.

5.6. ZUSAMMENFASSUNG

Ziel dieser Studie war es zu untersuchen, ob die Retention von Phosphor in der Tibia (TP) zur Schätzung der Phosphorretention im Ganzkörper (GKP) von Broilern verwendet werden kann. Hierfür kam eine auf Mais und Sojaextraktionsschrot basierende Basalration zum Einsatz (0,31% Gesamt-P). Wasserfreies Mononatriumphosphat oder wasserfreies Calciumhydrogenphosphat wurden stufenweise ergänzt, um den P-Gehalt im Futter um 0,08,

0,16 und 0,24% zu erhöhen. Das Verhältnis von Gesamt-Ca zu Gesamt-P lag in allen Rationen bei 2:1. Broiler der Herkunft Ross 308 (nicht geschlechtssortiert) wurden in Bodenhaltung aufgezogen. Am 11. und 25. Lebenstag wurden die Tiere den Rationen zugeordnet. Das Futter wurde *ad libitum* angeboten. Am 21. und 35. Lebenstag wurden die Tiere mittels CO_2 getötet und zwei Broiler pro Behandlung zufällig für die Ganzkörper- und Tibia-Analysen ausgewählt. Die restlichen Tiere wurden für eine weitere Studie verwendet. Die Tibien wurden isoliert und der Verdauungstrakt entleert. Die Ganzkörper einschließlich Federn und Eingeweiden wurden zerkleinert und anschließend getrennt von den Tibien verascht, um daraufhin den P- und Ca-Gehalt in beiden Fraktionen bestimmen zu können. Am 21. Lebenstag lag das Verhältnis von GKP/TP bei 21,3±1,3 (Mittelwert und Standardabweichung), am 35. Lebenstag bei 19,8±1,1. Der Anstieg der linearen Regression zwischen TP und GKP war für beide Lebensabschnitte identisch (17,7). Die Ergebnisse zeigen, dass Veränderungen im TP-Gehalt grundsätzlich geeignet sind, um Veränderungen in der P-Retention von Broilern zu schätzen.

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CHAPTER 6

COMPARISON OF TWO TYPES OF BASAL DIET FOR ASSESSING THE P AVAILABILITY OF A MINERAL P SOURCE IN BROILERS

6. COMPARISON OF TWO TYPES OF BASAL DIET FOR ASSESSING THE P AVAILABILITY OF A MINERAL P SOURCE IN BROILERS

6.1. ABSTRACT

The objectives of this study were (1) to determine the availability of a feed phosphate based on quantitative P retention by using a phytin-containing as well as a purified basal diet, and (2) to investigate the impact of supplemental inorganic P on inositol-6-phosphate (**IP_6**) hydrolysis. Corn-soybean meal (**SBM**)-based and purified basal diets were used (both with 1.8 g available P per kg dry matter). Anhydrous monosodium phosphate (**MSP_a**) was supplemented to increment the P concentration by 0.05%, 0.1%, and 0.15%. Titanium dioxide was used as the indigestible marker. Diets were pelleted through a 3-mm screen. A retention trial with excreta collection from d 20-24 was conducted (n=7 birds per diet). The level of P significantly affected P retention neither for the corn-SBM-based nor for the purified based diets ($P > 0.05$). However, the P level significantly decreased ($P = 0.015$) the IP_6 hydrolysis for the corn-SBM-based diets. Percentage P retention for MSP_a was calculated by linear regression analysis. P retention for MSP_a was 50% for the corn-SBM-based diets and 51% for the purified diets. The difference between the slopes for both types of diets was not significant ($P = 0.954$). It was concluded that there was no difference in P retention from MSP_a between corn-SBM-based and purified based diets if the calculation was based on linear regression analysis. However, it is suggested to use the corn-SBM-based diet for the evaluation of mineral P sources due to its similarity to commercial poultry feed.

6.2. INTRODUCTION

P is an extremely important nutrient in vegetable diets for chicks, and the bioavailability of the supplemental phosphate sources is critical for skeletal growth, BWG, and survival (Sullivan and Douglas, 1990). P supplements are expensive, making the concentration and availability of P in feedstuffs an important consideration in diet formulation (Veum, 2010). There are several possibilities to measure the P availability in poultry, but only retention and pc digestibility studies provide quantitative values that can be directly taken as the P availability. The ability to detect changes in Ca and P retention will provide important information needed to assess the economic value of increasing dietary concentrations of these key minerals and also to evaluate the effect of adjusting other nutrients on Ca and P utilization

(Coon et al., 2002). However, phytin is the principle storage form for phosphate in plant material and presents a nutritional obstacle for monogastric animals (Cowieson et al., 2011). A common misconception is that nonruminant animals lack endogenous phytase which is the reason for poor phytate disappearance (Adeola and Cowieson, 2011). Actually, poultry possess very effective phytase/phosphatase activity in the intestinal mucosa, blood, and liver and can readily dephosphorylate phytate into inositol and free phosphate for systemic distribution (Cowieson et al., 2011).

The outcome of a P availability study depends on many factors, and one of them might be the type of diet used. In experimental diets for poultry, mostly purified diets have to be used for assessing P digestion due to the fact that broilers are capable of hydrolyzing phytin P (Jongbloed and Kemme, 2002). This raises the question whether the results obtained from investigations with purified types of diets are valid for the phytin-containing diets used in practical feeding of broilers. Purified diets are also less palatable to poultry than practical-type corn-SBM diets (Sullivan and Douglas, 1990). Moreover, they exhibit other than corn-SBM pellet quality characteristics, which might considerably affect the results investigated with these types of diets (De Groote and Huyghebaert, 1997), because next to nutritional composition, pellet quality is often the most important factor of a complete feed (Loar and Corzo, 2011). There is also very few and inconsistent information on whether or not the inorganic P retention is changed by the presence of phytate in a diet.

Thus, the objective of this study was (1) to determine the availability of P from anhydrous MSP_a by using purified and practical-type basal diets based on quantitative P retention in 3-wk-old broilers, and (2) to investigate the impact of the inorganic phosphate level on the IP_6 hydrolysis of a corn-SBM-based diet.

6.3. MATERIAL AND METHODS

6.3.1. ANIMALS AND MANAGEMENT

Unsexed broiler hatchlings (Ross 308) were obtained from a local hatchery (Brüterei Süd, Regenstauf, Germany) and randomly allocated, 17 per pen, to 40 floor pens (154 × 154 cm) covered with pine shavings. The room temperature was reduced from 35 to 27.5 °C between d 1 and 7, from 27 to 24 °C between d 8 and 14, and from 23.5 to 22 °C between d 15 and 21 posthatch. In the rearing period (1 to 16 days of age), the chicks were provided free access to water and a commercial broiler starter diet (Deutsche Tiernahrung Cremer GmbH and Co. KG, Mannheim, Germany) containing 220 g CP, 12.5 MJ ME, 11.0 g of Ca, 5.5 g of P, and

500 FTU 3-Phytase (EC 3.1.3.8) per kg feed. A total of 20 hours of light were provided per day throughout the study. Mortality was recorded daily. The experiment was approved by the Animal Welfare Commissioner of the University in accordance with the Animal Welfare Regulations.

6.3.2. EXPERIMENTAL DIETS

Eight diets, based on two different basal diets, were formulated to meet or exceed the Gesellschaft für Ernährungsphysiologie (1999) requirements for all nutrients except for Ca und P. Main ingredients such as corn, SBM, potato protein, and cornstarch were chosen for Diet 1 (1.8 g available P (**aP**)/kg feed dry matter (**DM**)) (Table 21) to achieve a low P concentration and a low intrinsic phytase activity. Cornstarch, potato protein, cellulose, and sucrose were used for compounding the purified basal diet (Diet 2) to attain an almost phytin-free basal diet with a low P concentration (0.5 g P/kg feed DM) (Table 21). The content of aP in Diet 1 was calculated using the P retention value determined for a very similar corn-SBM-potato protein-cornstarch basal diet used in a previous study (Shastak et al., submitted). 1.8 g aP per kg feed DM in the purified basal diet was obtained by supplementation of MSP_a (Dr. Paul Lohmann GmbH KG, Emmerthal, Germany). The same MSP_a source was added to both basal diets to achieve 0.05%, 0.1%, and 0.15% of supplemental P in six further diets. Concentrations of Ca and P were calculated to be 0.68% and 0.34% per kg feed DM in Diet 1, and 0.52% and 0.26% per kg feed DM in Diet 2, respectively. P and Ca levels in the six other diets were adjusted by replacing cellulose and sand in the basal diets with respective levels of MSP_a and limestone. The Ca to P ratio in all experimental diets was calculated to be 2:1. Titanium dioxide was included as indigestible marker. The Na to Cl ratio of the two basal diets was adjusted by a variation of NaCl, $NaHCO_3$, and choline chloride using the molar masses of the elements. Diets were pelleted through a 3-mm die without using steam. The pellets of the purified diets were very hard in comparison to the corn-SBM-based diets, and therefore were partially fractured with a grain crusher (Eduard Engel e.U., Austria). Samples of all feed ingredients were analyzed for Ca and total P before compounding the experimental diets. Samples of pelleted diets were collected immediately before the beginning of the trial. They were ground through a 0.5-mm sieve of a grinding mill (Type ZM 1, Retsch GmbH, Haan, Germany) and stored at room temperature to await analyses. Analyzed concentrations of Ca and total P confirmed intended levels (Table 22).

Table 21. Ingredient composition of the basal diets (g/kg feed) and analyzed concentrations (g/kg dry matter)

Ingredient	Basal diet	
	Phytin-containing	Purified
Corn	542	-
Soybean meal, 51% CP	170	-
Potato protein, 75% CP	75	215
Potato protein, 70% CP	54	-
Cornstarch	85	491
Cellulose	-	93.7
Sucrose	-	80
Soybean oil	20	60
D,L-Methionine	1.5	3
L-Arginine	-	3
MSP_a	-	8.2
Exchange mixture[1]	39	30.8
Mineral mix	1[2]	1.5[4]
Vitamin mix	1.5[3]	1.9[5]
NaCl	1	0.8
Titanium dioxide	5	5
Choline chloride	2	4.6
Natrium bicarbonate	3	1.5
ME, MJ/kg calculated[6]	12.9	12.9
Analyzed concentrations (g/kg dry matter)		
Crude protein	238	197
Crude fat	60	74
Calcium	6.7	5.0
Total phosphorus	3.3	2.6[6]
IP_6-phosphorus	2.0	0.1

[1] Consisted of cellulose and sand (1:1), and limestone; P and Ca levels were adjusted by replacing of those components with three levels of the MSP_a as described in the text.

[2] Mineral mix [Celita SG 1,GFT MBH, Memmingen, Germany] provided per kg of complete Diet 1: Cu, 15 mg; I, 1.6 mg; Fe, 90 mg; Mn, 120 mg; Zn, 80 mg; Co, 0.6 mg, Se, 0.5 mg.

[3] Vitamin mix [Raiffeisen Kraftfutterwerke Süd GmbH, Würzburg, Germany] vitamin A, 9.000 IU; vitamin D_3, 2.250 IU; vitamin E, 22.5 IU; menadione, 1.8 mg; thiamine, 2.3 mg; riboflavin, 4.5 mg; niacin, 37.5 mg; calcium d-pantothenate, 10.5 mg; pyridoxine, 4.5 mg; vitamin B_{12}, 23 mcg; folic acid, 0.75 mg; biotin, 0.075 mg.

[4] Mineral mix [Celita SG 1,GFT MBH, Memmingen, Germany] provided per kg of complete Diet 1: Cu, 22.5 mg; I, 2.4 mg; Fe, 135 mg; Mn, 180 mg; Zn, 120 mg; Co, 0.9 mg, Se, 0.75 mg.

[5] Vitamin mix [Raiffeisen Kraftfutterwerke Süd GmbH, Würzburg, Germany] vitamin A, 11.400 IU; vitamin D_3, 2.850 IU; vitamin E, 28.5 IU; menadione, 2.3 mg; thiamine, 2.9 mg; riboflavin, 5.7 mg; niacin, 47.5 mg; calcium d-pantothenate, 13.3 mg; pyridoxine, 5.7 mg; vitamin B_{12}, 29.1 mcg; folic acid, 0.95 mg; biotin, 0.095 mg.

[6] 0.5 g P/kg DM from main ingredients; 2.1 g P/kg DM supplied by MSP_a.

Table 22. Calculated and determined concentrations of total P and Ca in the experimental diets (g/kg feed DM)

g/kg feed DM	Total P		Ca	
	Calculated	Determined	Calculated	Determined
BD[1] (Corn-SBM)	3.4	3.28	6.8	6.69
MSP_a (0.05 % P)	3.9	3.77	7.8	7.14
MSP_a (0.1 % P)	4.4	4.19	8.8	9.11
MSP_a (0.15 % P)	4.9	4.74	9.8	9.02
BD (Purified)	2.6	2.62	5.2	4.97
MSP_a (0.05 % P)	3.1	3.23	6.2	6.10
MSP_a (0.1 % P)	3.6	3.72	7.2	7.17
MSP_a (0.15 % P)	4.1	4.21	8.2	7.95

[1] BD=Basal diet.

6.3.3. RETENTION TRIAL

On d 16 posthatch, 56 chicks (with a BW of 620 ± 25 g) were moved individually into balance cages (45 cm wide × 50 cm deep × 42.5 cm high), allocated to 8 dietary treatments (7 chicks per diet), and received their respective diet *ad libitum* until d 19. Because of different feed intakes between the birds receiving basal diets 1 and 2, it was impossible to determine the specific mean *ad libitum* intake. Thus, from d 19 onward, the feed allowance was restricted to 80 g per bird and day. In spite of this restriction, voluntary feed intake was incomplete for one individual receiving the corn-SBM-based diets and for all birds receiving the purified diets; these refusals were recorded. The retention trial consisted of a 4-d lasting period of adaptation to the respective experimental diet and a 5-d lasting main balance period with restricted feeding and complete excreta collection. Feed was offered in two meals per day at about 0800 and 1700 h. From d 20 onward, excreta were collected once per day after morning feeding from metal pans underneath each cage during the 5-d period and frozen at -20 °C. Feathers were removed before each collection. Broilers were weighed individually on

d 16 and 24 posthatch. At the end of the collection period, excreta were defrosted, dried in a convection oven (Heraeus UT 6760, Hanau, Germany) at 65 °C for 4 d, and ground as explained for the feed.

6.3.4. CHEMICAL ANALYSES

Samples of feed and excreta were analyzed for DM (103 °C), P, Ca, and Ti. To determine the Ca, P, and Ti concentrations, a modification of method 10.6.1 (Verband Landwirtschaftlicher Untersuchungs- und Forschungsanstalten, 2006) was used for wet digestion. Duplicates for each sample were weighed (0.5 g) and transferred into glass digestion flasks. Then, 20 mL sulphuric acid (95-97% (w/v), product no. 1650.2500, for analysis, NeoLab Migge GmbH, Heidelberg, Germany) and 2.5 mL nitric acid (65% (w/v), product no. 865.2500, for analysis, Th. Geyer GmbH & Co. KG, Renningen, Germany) were added. The flasks were subsequently heated from 100 to 200 °C for 30 min in a block digestion system (Behr K 20 L, Behr Labor-Technik GmbH, Düsseldorf, Germany). After cooling, 2.5 mL of nitric acid were added, and the solutions heated again from 225 to 300 °C for 75 min. After returning to room temperature, the solutions were transferred into 250-mL Erlenmeyer flasks, filled up with double distilled water, and filtered through paper filters (ashless MN 615 w, product no. 090304, Macherey-Nagel GmbH & Co. KG, Düren, Germany) into 100-mL plastic bottles. As suggested by Boguhn et al. (2009), Ti concentrations in the solutions were determined together with Ca and P concentrations using an inductively coupled plasma optical emission spectrometer (VISTA PRO, Varian Inc., Australia) and specific wavelengths for each element (Ca, 317.933; P, 213.618; and Ti, 334.941). Feed samples were also analyzed for CP (method 4.1.1) and crude fat (method 5.1.1) (Verband Landwirtschaftlicher Untersuchungs- und Forschungsanstalten, 2006).

For IP_6 analysis, samples were extracted with a solution of 0.2 M ethylendiamintetraacetic acid, 0.1 M NaF, pH 10. Sample clean-up was done with centrifugal ultrafiltration using a Microcon filter (cut-off 30 kDa) devise (Millipore, Bedford, MA, USA); centrifugation was done at 14 000 x g for 30 min at 4 °C. Filtrates were analyzed by high-performance ion chromatography (HPIC) and UV detection at 290 nm after postcolumn derivatization using an ICS-3000 system (Dionex, Idstein, Germany) equipped with a Carbo Pac PA 200 column and corresponding guard column. $Fe(NO_3)_3$ solution in $HClO_4$ was used as reagent for derivatization, as described by Philippy and Bland (1988).

6.3.5. CALCULATIONS

The retention of P (y) was calculated for each bird according to the following equation:

$y\ (\%) = 100 - 100 \times [(TiO_{2\ diet} \times P_{\ excreta}) / (TiO_{2\ excreta} \times P_{\ diet})]$

where $TiO_{2\ diet}$ and $TiO_{2\ excreta}$ = analyzed concentrations of TiO_2 in the diet and excreta samples (g/kg), and P_{diet} and $P_{excreta}$ = analyzed concentrations of P in the diet and excreta samples (g/kg). The retention of Ca and IP_6 hydrolysis were calculated accordingly, using analyzed Ca and IP_6 concentrations. The retention of P and Ca as well as IP_6 hydrolysis were also calculated by using quantified amounts of feed intake and excreta.

The retention of the mineral P source was obtained from the slope of linear regressions of the type $y = a + mx$, calculated between the level of added inorganic P (g/kg feed DM) (x) and the retained amount of P (g/kg feed DM) (y). The calculated slope (m) multiplied by 100 is the percentage retention of the P source. By this regression, the response to the P source was separated from the P contained in the basal diet, and corrections of specific endogenous losses were not necessary. Calculations were made using data for the basal diet and three levels of added P from MSP_a. The number of data sets used in regression analysis for each P source was 28. Regressions were calculated using GraphPad Prism 5.0 (Graph Pad Software, Inc.).

6.3.6. STATISTICAL DATA ANALYSES

The individual bird was the experimental unit in the retention trial. ANOVA was performed to analyze data from the experiment. All data were analyzed using the procedure for linear mixed models (PROC MIXED) of the software package SAS for Windows (version 9.1.3, SAS Institute Inc., Cary, NC, USA). The analysis was done according to the following model:

$y_{ik} = \mu + \alpha_i + e_{ik}$

where y_{ik} is the parameter, μ is the overall mean, α_i is the treatment, and e_{ik} is the error term.

6.4. RESULTS

P retention for the corn-SBM-based basal diet was 48.0% (Table 23). No significant effect of the P supplementation level was detected. The addition of 0.05%, 0.1%, and 0.15% P from MSP_a to the basal diet resulted in P retention values of 50.4%, 48.3%, and 49.1%, respectively. The IP_6 hydrolysis for the basal diet was 70.4%. The P level had a significant effect ($P = 0.015$) on the IP_6 hydrolysis for the corn-SBM-based diets. For the 0.05%, 0.1%,

and 0.15% P MSP_a supplemented diets, IP_6 hydrolysis values declined to 61.0%, 52.9%, and 39.5%, respectively.

Table 23. Results of retention trials (n=7 broilers per treatment) for birds fed corn-SBM-based diets

	BD	MSP_a			Pooled	*P*
		0.05%	0.1%	0.15%	SEM	(ANOVA)
BWG d 16-24, g	318	325	315	260	22.7	0.181
Feed intake d 19-23, g	394	399	399	319	8.9	0.473
P intake, mg/d	231	269	298	323	7.5	<0.001
P excretion, mg/d	116	122	145	156	6.7	<0.001
P retention[1], %	48.0	50.4	48.3	49.1	2.3	0.886
Ca intake, mg/d	470	509	649	614	14.2	<0.001
Ca excretion, mg/d	418	425	466	454	16.7	0.163
Ca retention, %	7.8	8.4	23.7	21.9	3.3	0.002
IP_6 hydrolysis, %	70.4	61.0	52.9	39.5	6.3	0.015

[1] Retention of P and Ca, and IP_6 hydrolysis are expressed in % of intake. Excreta were collected from d 20 - 24, retention and IP_6 hydrolysis were calculated based on the marker technique.

P retention for the purified basal diet was 70.4% (Table 24). There was no significant effect of the P supplementation level. The addition of 0.05%, 0.1%, and 0.15% P from MSP_a to the basal diet resulted in P retention values of 71.9%, 62.6%, and 64.6%, respectively.

Table 24. Results of retention trials (n=7 broilers per treatment) for birds fed purified diets

	BD	MSP_a			Pooled	*P*
		0.05%	0.1%	0.15%	SEM	(ANOVA)
BWG d 16-24, g	102	116	89	106	9.9	0.293
Feed intake d 19- 23, g	280	298	271	268	11.9	0.293
P intake, mg/d	133	175	183	205	7.7	<0.001
P excretion, mg/d	32	39	57	64	4.8	<0.001
P retention, %	70.4	71.9	62.6	64.6	2.8	0.078
Ca intake, mg/d	253	330	353	387	14.6	<0.001
Ca excretion, mg/d	141	156	184	208	14.1	0.012
Ca retention, %	32.7	40.8	36.9	39.2	3.0	0.277

[1] Retention of P and Ca are expressed in % of intake. Excreta were collected from d 20 - 24, retention was calculated based on the marker technique.

The Ca retention was significantly improved for the corn-SBM-based diets (P = 0.002) when the P concentration was increased (Table 23). The Ca retention for the BD was 7.8%. The addition of 0.05%, 0.1%, and 0.15% P from MSP_a to the basal diet resulted in Ca retention values of 8.4%, 23.7%, and 21.9%, respectively. The Ca retention for the purified basal diet was 32.7%. No significant effect of the P supplementation level was detected (P = 0.277). The addition of 0.05%, 0.1%, and 0.15% P from MSP_a to the basal diet resulted in Ca retention values of 40.8%, 36.9%, and 39.2%, respectively.

Both Ca and P intake, as well as P excretion, were significantly affected by the level of supplemental P (P < 0.001) for corn-SBM-based diets. However, the P level had no significant effect on the Ca excretion (P = 0.163) (Table 3). The P level had a significant effect (P < 0.05) on the Ca and P intake/excretion for purified diets (Table 24).

The relationship between P intake and retained P was linear for both diets (Figure 7). The retention of P from MSP_a, determined by linear regression analysis, was 50% and 51% for corn-SBM-based and purified diets, respectively. The differences between the slopes of linear regressions of Diet 1 and 2 were not significant (P = 0.954).

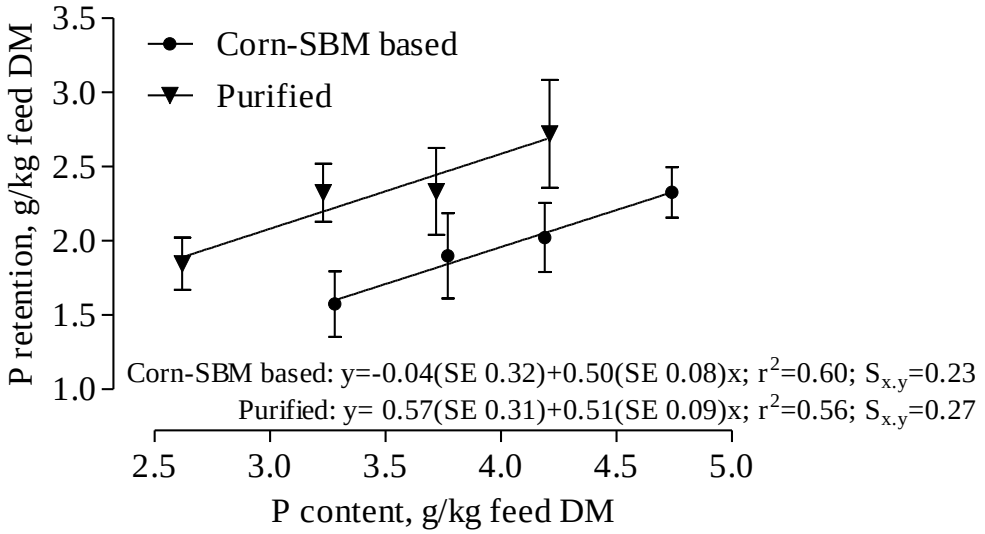

Figure 7. P retention depending on P content of diet in 3-wk-old broilers

6.5. DISCUSSION

The results of this experiment demonstrate that there was no difference in P availability from MSP_a calculated on a linear regression basis for both types of diets (Figure 7). The slopes investigated for MSP_a were 0.50 for Diet 1 and 0.51 for Diet 2. The difference between the slopes (0.50 ± 0.08 vs. 0.51 ± 0.09) was not significant (P = 0.954). Hence, it was possible to calculate one slope for both diet types. The pooled slope for MSP_a equaled 0.50. Sullivan (1999) also reported that biological value results obtained with purified and practical-type diets were equally reliable. However, mostly bone ash or BWG were used as response criteria.

Wilcox et al. (1954) and Wilcox et al. (1955) investigated the availability of P from different sources for young turkeys fed purified and practical-type diets using BW, bone ash, and mortality as response criteria. Despite different inclusion rates of test phosphates in the purified and practical-type diets, similar availability values were reported for both types of diets for monobasic calcium phosphate (97 vs. 101%, respectively), tribasic calcium phosphate "N.F. IX" (73 vs. 72%, respectively), beta tricalcium phosphate (60 vs. 69%, respectively), and DFP "A" (75 vs. 82%, respectively). The authors concluded that the results of the experiment with practical-type diets agreed reasonably well with the results obtained when a purified diet was used, but probably gave a more accurate indication of the possible value of the various products under commercial conditions. Nelson and Peeler (1961) also reported that the biological value of phosphates obtained with a purified diet could be applied to practical diets.

The P retention value of 50% for MSP_a in the present experiment is lower than that reported previously: 70 and 63% for 3- and 5-wk-old broilers, respectively, for the same phosphate source (Shastak et al., submitted). Although Diet 1 in this trial was similar to the basal diet used in the previous study, other batches of ingredients were used for compounding the basal diet as well as another batch of MSP_a. There were also other factors, for example the age of the animals, which might have affected the results. The birds were 16-d-old in comparison to 11- and 25-d-old chicks employed in the previous experiment. Furthermore, we chose the heaviest birds from the flock on d 16 posthatch (620 ± 25 vs. 240 ± 15, 1150 ± 50 after average weight of the flock in the previous experiment) in order to decrease the possibly severe effect of P deficiency and to minimize mortality owing to a poor consumption of purified-type diet, as reported in the literature (Dewar and Downie, 1984; Dewar, 1986; Wedekind et al., 1992; Sullivan and Douglas, 1990). The differences in feed intake and BWG between the birds that consumed Diet 1 and 2 in our experiments confirm this concern. Moreover, there was a difference in the phosphate inclusion level (0.05 vs. 0.08%) between the present and the previous study. Additionally, the feed allowance was calculated differently. These factors taken together at least in part might have led to different retention values obtained for the same mineral phosphate. Such variation in the availability of the same phosphate under various experimental conditions may be of disadvantage for formulating adequate availability tables for practical feeding. Attempting to determine a simple, unique bioavailability value of a source applicable under all conditions can be considered somewhere between frustrating and misleading (Miles and Henry, 2000), which is also certainly applicable for phosphates. Experimentation is not perfect; sometimes, unknown factors will radically influence results and accepting results of all experiments may lead to costly errors in diet formulation (Miles and Henry, 2000). Thus, a defined standardized specification for a system of P evaluation is an absolute necessity for obtaining reliable availability numbers for broiler feeding.

It is possible to measure P availability under standardized conditions as described by Van der Klis and Versteegh (1996). Purified P deficient diets (1.8 g available P/kg feed), in which the major part of the dietary P originates from the test feedstuff, were used. This methodology allows a precise measurement of P availability not only for different feed phosphates but also for other feedstuffs. A value of 92% was reported for MSP × H_2O by these researchers. In our study, for the purified basal diet (1.8 g aP/kg feed DM or 1.6 g aP/kg feed) and the first inclusion level of 0.05% P from MSP_a (2.3 g aP/kg feed DM or 2.1 g aP/kg feed), we obtained a total P retention of 70.4% and 71.9%, respectively. In general, the wider

the ratio of test element to basal diet element, the more sensitive the test for measuring bioavailability (Ammerman, 1995). The contribution of aP from the basal diet to the total aP content of these two diets was 19% and 15% on DM basis, respectively, which was only a little higher than defined by Van der Klis and Versteegh (1996). Nevertheless, if the P contribution of the basal diet was not taken into consideration and the total retention of the diet was not only ascribed to MSP_a, the value was about 20% units higher than that determined by linear regression analyses (70.4% and 71.9% vs. 51%). Thus, the method of the calculation of P retention values is crucial to the interpretation of the obtained results. Payne (2005) conducted three balance trials using two purified diets and the same principle as described by Van der Klis and Versteegh (1996) to investigate P retention values from different inorganic phosphates in broilers. The author reported a value of 27% for MSP × H_2O in the first experiment (3.5 g aP/kg diet), 47% and 41% in the second experiment (3.5 and 2.0 g aP/kg diet, respectively), and 47% in the third experiment (3.5 g aP/kg diet). However, the purified diets were offered as mash and thus feed intake was low.

We obtained 70.4% IP_6 hydrolysis for the corn-SBM-based basal diet in this experiment. Such high values for PP hydrolysis were also reported for broiler chickens fed corn-SBM basal diet. A value higher than 50% was determined by Edwards (1983). Mohammed et al. (1991), Edwards (1993), and Mitchell and Edwards (1996) showed that chicks are able to hydrolyze more than 70% of PP determined on the basis of excreta collection. Harms et al. (1962) and Waldroup et al. (1964) reported that phytic acid was as available to chicks as the P from dicalcium phosphate. Studies with chickens indicate that PP utilization is variable and that dietary factors including the level of Ca, nPP, total P, and vitamin D, as well as feed processing and feed or ingredient particle size, may influence PP hydrolysis in the gastrointestinal tract (Angel et al., 2002). One of the most important factors is the Ca content in a diet. Applegate et al. (2003) showed that intestinal phytase activity was 9% greater in broiler chicks fed 4 vs. 9 g/kg Ca and apparent ileal PP hydrolysis was 11.9% greater. Tamim and Angel (2003) and Tamim et al. (2004) showed that broilers were able to hydrolyze more than 65% of PP determined at the end of the ileum if no Ca was added to a corn-SBM-based basal diet. A strong dependence on the Ca level for phytate degradation in the lower part of the intestine was also pointed out by Sandberg et al. (1993) and Schlemmer et al. (2001) in pigs, and by Nahapetian and Young (1980) in rats. Motzok (1963) and McCuaig et al. (1972) found that excess Ca could also reduce alkaline phosphatase activity of the chick intestine.

A number of factors have to be considered when discussing phytate degradation in the gut, e.g., if there is an effect of dietary phytase, intestinal mucosa phytase, or phytase produced by the intestinal microflora (Sandberg, 2002). Because our, mostly corn-SBM-based basal diet might have only low intrinsic phytase activity, the hydrolysis of IP_6 had to be caused by phytases from the gut microflora or intestinal mucosal phytases. Phytate hydrolysis activity was demonstrated in the extracts of the mucosae of the small intestine of the rat, chicken, calf, and man (Bitar and Reinhold, 1972). However, there was a Ca:P ratio of 2 to 1 in our trial for all diets used, which might have complicated the hydrolysis of IP_6 in the small intestine. It was shown that the large intestine plays an important role in the PP hydrolysis in monogastrics (Wise et al., 1983; Lantzsch et al., 1988; Sandberg et al., 1993; Skoglund et al., 1997; Schlemmer et al., 2001, Sandberg, 2002). Kerr et al. (2000) showed in broilers and laying hens that levels of IP_6 and lower derivates were very low in the ceca, indicating rapid and nearly complete IP_6 hydrolysis by cecal microorganism phytase. The authors concluded by comparison of the ileal and fecal IP_6 disappearance, that hindgut microorganisms had an important impact on the IP_6 hydrolysis.

Shastak et al. (submitted) used a similar basal diet with the Ca:P ratio of 2:1. The authors demonstrated that the pc digestibility of Ca was almost 3-fold greater than respective Ca retention values determined in the balance trials with the same age and flock of broilers, meaning that the great part of pc-digested Ca was excreted with urine. Therefore, regarding the present experiment, it can be suggested that the great part of IP_6 hydrolysis occurred in the cecal-rectum region due to more convenient conditions for hydrolysis owing to a lower Ca content in this region in comparison to the small intestine.

The supplementation of MSP_a significantly affected the IP_6 hydrolysis in the present experiment (Table 23). The increment of the P level from MSP_a decreased the IP_6 hydrolysis from 70.4% for unsupplemented diets to 39.5% for 0.15% supplemented diets. These results are in agreement with what Van der Klis and Versteegh (1996) and Manangi and Coon (2006) found in broilers, reporting that supplementation with inorganic phosphate decreased phytate breakdown from the diets fed. Onyango et al. (2001) demonstrated that there was no significant difference in phytase activity in the duodenum, jejunum, and ileum of broiler chicks fed a P-adequate diet and those fed a low-P diet. In contrast, McCuaig and Motzok (1972) reported that low dietary inorganic P (0.16%) increased the intestinal alkaline phosphatase activity by 50% compared to adequate inorganic P (0.48%). Nevertheless, it was shown by Shieh et al. (1969) that the synthesis of the enzymes (phytase and phosphohydrolase) by *Aspergillus ficuum* was repressed by a high orthophosphate

concentration in the fermentation medium. Orthophosphate was recognized as a competitive inhibitor of enzymatic phytate degradation and a depressing factor of bacterial phytase production *in vitro* (Konietzny and Greiner, 2002). Thus, regarding our experiment, it seems that supplemental phosphate might have primarily affected the microorganism's production of phytase in the cecal-rectum region if it had been the main site of IP_6 hydrolysis. Because there is possibly barely any absorption of phosphate in the large intestine, the total P retention values remained the same for all supplemental levels of P (Table 23). However, there is still a possibility that IP_6 hydrolysis could also take place in the proximal parts of the gastrointestinal tract (crop, proventriculus, and gizzard). In this case, the released phosphate would affect the investigated slope (availability) for supplemented phosphate as it could be absorbed in the small intestine. Moreover, the increasing Ca:PP ratio with every supplementation P level could be a reason for the decline in IP_6 hydrolysis too. However, all of this cannot be verified through this experiment.

It can be concluded that there was no difference in P retention from MSP_a between corn-SBM and purified basal diets. The IP_6 hydrolysis declined along with the increase in the MSP_a inclusion rate into the corn-SBM-based diet. However, it might be suggested to use the corn-SBM-based basal diet in the evaluation of mineral P sources due to its similarity to commercial poultry feed where phytate-containing diets are widely used.

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CHAPTER 7

GENERAL DISCUSSION

7. GENERAL DISCUSSION

Due to the sharp increase in the prices for feed phosphates and environmental concerns associated with excessive excretion of P by livestock, the knowledge about the availability of P from mineral sources has gained in importance in the last decade. P in plant ingredients, which are the basis for compound poultry feed, is largely present in the form of phytic acid and its salts (Eeckhout and de Paepe, 1994), and their availability in poultry is incomplete. Failure to supply adequate amounts of P may lead to severe consequences in terms of reduced performance, increased condemnations, excessive mortality, and reduction in carcass quality (Waldroup, 1999). Mineral phosphate supplementation is needed to increase the concentration of aP in the diet and meet a bird's requirement. Therefore, it is necessary to evaluate mineral P sources regarding their availability to poultry in order to meet their P requirements and to avoid excessive supply and P excretion.

There are various approaches which are used by different laboratories for the determination of P availability. The main problem is, however, that it is not clear how the differences between approaches affect the results. The development of a standardized method for P evaluation which allows obtaining quantitative values of P availability is the basis for optimizing the dietary P concentration in broiler diets. In the following, the potential of different response criteria in P evaluation for poultry will be discussed.

7.1. DEVELOPMENT OF A STANDARD TEST FOR P EVALUATION

It has become increasingly clear that retainable P values for individual feedstuffs are needed in order to allow poultry meat and egg producers to make feed formulation decisions about feed P and commercial phytase based on the economical value of products compared to the economic disadvantages of P in poultry waste (Coon et al., 2002). The determination of exact quantitative values of the P availability in different plant and mineral P sources will make it possible to formulate diets which are closer in their aP content to the needs of a bird while minimizing or avoiding excess supplementation. Thus, it is necessary to develop a common method for determining P availability in broiler experiments. The biological value of the particular P source under study, included in a given diet, may not be the same when measured in another diet, due to interactions between other nutrients (Gueguen, 1999). The differences in the availability (retention) for the same mineral P source between the different trials (Chapter 3 vs. Chapter 6) conducted within this thesis indicate the imperative for setting

standardized experimental conditions for the assessment of the aP content in various feed phosphates. The prime factors to standardize should be (1) response criterion; (2) age and strain of broilers; (3) composition of the basal diet, namely, levels of P, Ca, vitamin D, and phytate content; (4) period of adaptation and duration of experimental feeding. By using several supplementation levels of a given phosphate source, the birds have to be fed with a slight restriction in the amount of feed to ensure that differences in P intake originate only from the supplemented mineral P sources. Such feed restriction for pc digestibility tests is, however, very difficult to implement. The experimental diets should be fed in a pelleted form, because mash form does not play a big role in the practical feeding of growing birds. However, other aspects may also be taken into consideration. As shown by Edwards and Gillis (1959), a growing chick tends to overcome a history of P depletion by retaining a greater percentage of the intake if higher quantities become available. Ashwell and Angel (2010) reported that when broiler chicks were challenged with a diet low in P for 90 h posthatch, they obtained the ability to better utilize P later in life. For instance, the reduction of P in the posthatch diet had a significant effect on the expression of the intestine-specific Na/P-cotransporter by stimulating an average 2.8-fold increase in the mRNA levels in the small intestine. This data provided the first evidence for neonatal programming of gene expression in an oviparous species. Therefore, using a standardized starter diet may be also of advantage. From an applicability standpoint, the use of aP requirement recommendations would have to be based on an extensive database that contains ingredient averages as well as range and standard deviations (Angel, 2006). For this, however, an exact definition for "available P" and other terms has to be established. Suggestions for definitions were given by Rodehutscord (2009) and by a group of experts who work on a standard trial protocol (WPSA[4] in preparation).

One of the most important factors that can affect results is the Ca:P ratio in the diet. Some researchers used a constant Ca content at different P levels in the diets, while others increased Ca along with P; the Ca:P ratio might be specified as either Ca:nPP or Ca:aP or Ca:total P ratio. This makes the results barely comparable between experiments. The usage of the same specification for experiments is of great importance for a standardization of P evaluation.

The most data on the availability of P from various inorganic feed phosphates are available for broilers. There is also some information available for young turkeys, but only

[4] Working Group 2 – Nutrition – of the European Federation of branches of the World's Poultry Science Association (Sub-Committee on the mineral requirements of poultry).

little data available for growing ducks. It is questionable if the values obtained in studies with broiler chicks could be applied for other poultry species. Grimbergen et al. (1985) assessed the apparent pc digestion of P of inorganic feed phosphates in a corn-SBM-based diet for young turkeys. The feed phosphates were added to a low P basal diet at four levels (1, 1.5, 2, and 2.5 g added P per kg feed). The authors reported that the mean values of four P levels pc digested for DCP_a, DCP × H_2O, and MCP were 34.7%, 41.1%, and 47.4%, respectively. Using the same criterion, Ketels and De Groote (1988) reported estimates of pc digestibility for DCP_a, DCP × H_2O, and MCP sources fed to male broilers of 1 to 21 days of age to be 67%, 73%, and 71%, respectively. In their study, a basal diet, which was poor in available P, was supplemented with six increasing amounts of P (0.1%, 0.15%, 0.20%, 0.25%, 0.35%, and 0.45% of added P). Rodehutscord et al. (2003), using balance approach and nonlinear regression analysis, reported a P availability of 75% and 73% for MCP in 17- and 35-d-old ducks. The pc digestibility for MSP_a and DCP_a sources obtained in our study (Chapter 3) were 67% and 30% in 3-wk-old broilers, respectively. The differences in the availability of inorganic feed phosphates based either on P pc digestibility or on P retention between the studies suggest that estimates can vary considerably between laboratories and species. However, there were, besides the poultry species factor, discrepancies in experimental conditions and methods employed. Therefore, the data may not be comparable if using the values from different experiments. Hence, Rodehutscord and Dieckmann (2005) studied whether the availability of P was different among poultry species. The researchers used the same basal diets and experimental conditions for all poultry species tested. They stated that inorganic P sources could be expected to be different in their availability between broilers, turkeys, and ducks. Among the species studied, ducks were most efficient in utilizing inorganic P. The authors concluded that with regard to feed compounding, availabilities of inorganic P sources determined with broilers can be applied for turkey and duck feeds without a risk of overestimating availabilities. Quails could be used as model species for broilers in P availability studies, but the dietary P levels need special adjustment. Therefore, in order to obtain exact values of P availability, it seems to be reasonable to evaluate inorganic feed phosphates separately for each poultry species. Otherwise, it will certainly lead to P over- or under-supplementation for some of them.

The inorganic P supplementation level seems to be a crucial factor in determining P availability values of a given feed phosphate. Edwards and Gillis (1959) already showed that a chick utilizes P very efficiently as long as the amount in the diet does not exceed the requirement. Otherwise, P utilization is reduced. Leske and Coon (2002) demonstrated that

the P retention from different P sources is dependent on the amount of the P source included into a corn-SBM-based diet. Thus, the data obtained with purified types of diets under marginal P supply might be barely repeatable under commercial conditions. It can thus be concluded that the values obtained with purified diets with one supplementation level might be overestimated. However, the usage of several inclusion rates in a bioassay covers a wider range of P supply with the advantage of the assessment of availability under different suboptimal P levels and thus might be much closer to the practical conditions. Additionally, this implies that the usage of linear regression analyses and thus a correction of basal endogenous P losses are not necessary. The results of the trial in Chapter 6 of this thesis confirm this assumption. While P retention values for the two lower inclusion levels of MSP_a in the purified type of diet were about 70%, the retention value calculated on the basis of linear regression analyses was 20% units lower. Therefore, the absolute or true P utilization or availability data are very specific for a given set of experimental conditions, which is why some limits on their practical application should be taken into consideration (McGillivray, 1978; De Groote and Huyghebaert, 1997).

Although ionic Ca forms one of the weaker complexes with phytic acid, Ca phytates are among the most common insoluble phytate complexes in the digestive tract because Ca is present in most diets in the highest quantity among all the mineral elements (Soares, 1995). The results of the trial in Chapter 6 show that at least under suboptimal P supply, broiler chickens may hydrolyze a great amount of IP_6 in the GIT even at a Ca:P ratio of 2:1. Van der Klis and Versteegh (1996) pointed out that the values for PP degradation under standardized experimental conditions will be maxima in practice. However, if the most of IP_6 hydrolysis occurred in the cecal-rectum region, it possibly had no or hardly any effect on the total P retention of the diet. From this point of view, it becomes clear that a differentiation in PP and nPP is unsatisfactory if using P retention as a method for a standardized test. The reason for this is that it is unclear how much PP was actually retained, because it cannot be defined whether nPP in excreta originated from IP hydrolyzed in the integrative segment or was not retained from the feed. This problem may be overcome by the usage of pc digestibility. Thus, the issue of IP hydrolysis in different parts of GIT needs detailed clarification in order to be able to make feed formulation correspond more exactly to the P requirements of broilers.

7.2. RELATIVE BIOLOGICAL AVAILABILITY VS. RETENTION OR PC DIGESTIBILITY IN P EVALUATION

Different criteria of P evaluation were estimated in 3- and 5-wk-old broilers in the trials of this thesis (Chapter 3, 4, 5, and 6). Relative availability values based on bone criteria caused different rank orders for mineral P sources in comparison with retention data in the studies presented. However, this does not mean that the bone criteria are not sensitive enough in P evaluation. As a matter of fact, bone ash is a good criterion to determine Ca and P adequacy (Nelson and Walker, 1964; Nelson, 1967; Angel, 2006). Moreover, different bone ash criteria also provide similar results in the estimation of P availability (Chapter 4). The Pearson correlation coefficients (r) for different bone criteria evaluated in this study (Chapter 4) are presented in Tables 25 and 26.

Table 25. Pearson correlation coefficients between all bone criteria evaluated in Period 1

Criterion[1]	TBA, mg/bone	TMTA, mg/bone	TOA, mg	FA, mg	TD, mg/cm^3	CD, mg/cm^3	CC, mg/mm	SSI-P, mm^3	TBA, %	TMTA, %	TOA, %
TBA, mg/bone	-	0.983	0.968	0.981	0.882	0.862	0.969	0.964	0.771	0.818	0.608
TMTA, mg/bone	0.983	-	0.985	0.998	0.910	0.892	0.961	0.959	0.806	0.804	0.620
TOA, mg	0.968	0.985	-	0.993	0.916	0.906	0.957	0.962	0.792	0.812	0.666
FA, mg	0.981	0.998	0.993	-	0.917	0.903	0.965	0.965	0.803	0.813	0.639
TD, mg/cm^3	0.882	0.910	0.916	0.917	-	0.975	0.918	0.936	0.708	0.835	0.634
CD, mg/cm^3	0.862	0.892	0.906	0.903	0.975	-	0.921	0.929	0.701	0.803	0.627
CC, mg/mm	0.968	0.961	0.957	0.965	0.981	0.921	-	0.990	0.738	0.837	0.625
SSI-P, mm^3	0.964	0.959	0.962	0.965	0.936	0.929	0.990	-	0.744	0.837	0.633
TBA, %	0.771	0.806	0.792	0.803	0.708	0.701	0.738	0.744	-	0.565	0.594
TMTA, %	0.818	0.804	0.812	0.813	0.835	0.803	0.837	0.837	0.565	-	0.693
TOA, %	0.608	0.620	0.666	0.639	0.634	0.627	0.625	0.633	0.594	0.693	-

[1]TBA=tibia ash; TMTA=tarsometatarsus ash; TOA=toe ash; TD=total density; CD=cortical density; CC=corticalis content; SSI-P= Strain Strength Index.

Table 26. Pearson correlation coefficients between all bone criteria evaluated in Period 2

Criterion[1]	TBA, mg/bone	TMTA, mg/bone	TOA, mg	FA, mg	TD, mg/cm^3	CD, mg/cm^3	CC, mg/mm	SSI-P, mm^3	TBA, %	TMTA, %	TOA, %	BBS, N
TBA, mg/bone	-	0.968	0.944	0.968	0.624	0.557	0.948	0.916	0.809	0.674	0.520	0.570
TMTA, mg/bone	0.968	-	0.963	0.997	0.634	0.576	0.906	0.873	0.787	0.617	0.459	0.536
TOA, mg	0.944	0.963	-	0.980	0.600	0.543	0.865	0.836	0.748	0.575	0.420	0.521
FA, mg	0.968	0.997	0.980	-	0.633	0.575	0.901	0.867	0.781	0.614	0.456	0.536
TD, mg/cm^3	0.624	0.634	0.600	0.633	-	0.963	0.601	0.421	0.748	0.621	0.388	0.565
CD, mg/cm^3	0.557	0.576	0.543	0.575	0.963	-	0.554	0.339	0.725	0.577	0.363	0.546
CC, mg/mm	0.948	0.906	0.865	0.901	0.601	0.554	-	0.948	0.762	0.620	0.496	0.596
SSI-P, mm^3	0.916	0.873	0.836	0.867	0.421	0.339	0.948	-	0.688	0.512	0.411	0.539
TBA, %	0.809	0.787	0.748	0.781	0.748	0.725	0.762	0.688	-	0.624	0.424	0.633
TMTA, %	0.674	0.617	0.575	0.614	0.621	0.577	0.620	0.512	0.624	-	0.864	0.339
TOA, %	0.520	0.459	0.420	0.456	0.388	0.363	0.496	0.411	0.424	0.864	-	0.186
BBS, N	0.570	0.536	0.521	0.536	0.565	0.546	0.596	0.539	0.633	0.339	0.186	-

[1]see Table 25; BBS=bone breaking strength.

As expected, all bone criteria were positively correlated in Period 1 and 2. The closest correlations (r > 0.94 for both periods) were obtained between bone ash weights of tibia, tarsometatarsus, and toes, meaning that they were equally sensitive in the assessment of P availability in broilers. There was a high strength (r > 0.86) of the linear association between tibia, tarsometatarsus, and toe bone ash weights and the QCT measurements in Period 1. This demonstrates that QCT measurements might be also used as a tool to predict bone mineralization in broilers. However, with aging (Table 26) the association of bone ash weight criteria with QCT measurements decreased. Because the bones of older birds have a higher fat content, an additional error could occur in the QCT measurements, which might be seen as a disadvantage of the single-energy X-ray QCT system. Thus, it can be suggested that the single-energy QCT measurements are preferable in the first three weeks of age, as they can provide a very solid estimation of mineralization and mechanical properties of bones in this age period. Noticeable is a rather poor correlation between absolute value (bone ash weight) and relative value (bone ash concentration (%)) in comparison to other bone criteria in both age periods. Huyghebaert et al. (1980), Hall et al. (2003), and Coon et al. (2007) also reported that tibia ash weight was a more sensitive criterion in P evaluation than tibia ash concentration (%). Therefore, tibia, tarsometatarsus, and toe ash concentration (%) seem to be rather undesirable in the P evaluation for broilers.

The application of bone criteria has been the basis for the assessing of the quality of feed phosphates since the 1940s. However, the relative biological availability RBA data based on bone criteria show that there is a great variation not only in P availability between different feed phosphates, but also between the same inorganic feed phosphate and their batches, which is similar to the quantitative data obtained in the balance and pc digestibility trials described in this thesis. The variation in P availability from plant products and commercially used P supplements can be quite large (Soares, 1995). Waibel et al. (1984) studied the extent of variation in P availability of commercial P supplements using a slope ratio bioassay in young turkeys. One of the MDCP examined had a low availability in the first experiment (76.7%), but provided a value of 105.5% in the second experiment relative to a MDCP reference standard. It must be emphasized that the corn-SBM-based diet and experimental conditions were the same for both trials. Altogether, the variation in the availability for 7 commercial MDCP, 20 DCP, and 20 DFP relative to a MDCP reference standard in the first trial showed a 31.8, 31.2, and 17.6 percentage units' difference using tibia ash as response criterion. Therefore, the variation in biological availability within the same sources can be high. It is also clear that such variation would make a considerable difference in the availability of P in a

complete commercial corn-SBM-based diet. Similarly, Lima et al. (1997) found that the availability of P determined by the slope ratio method in 7 different commercial DCP relative to pure calcium phosphate dibasic dihydrate ($CaHPO_4 \times 2H_2O$) as calculated from tibia ash percentage and tibia breaking strength ranged from 80.3 to 107.8% and from 79.3 to 110.5%, respectively. However, commercial DCP usually contain varying amounts of dicalcium phosphate (hydrous and anhydrous forms), monocalcium phosphate, phosphoric acid, and impurities, depending on the origin of the row material and procedures employed in their industrial production (Lima et al., 1995). Therefore, these differences in the availability of various DCP could be partially attributed to different contents of hydrous and anhydrous forms in commercially available DCP sources.

Other response criteria such as bone mineral density or breaking strength may also be applied as precise, accurate, and rapid indicators of bone quality in biological assays of P evaluation. However, they have the same disadvantages as bone ash: It is possible to obtain only relative values of P availability. Usually, a poor-in-available-P basal diet, based mostly on corn-SBM, is supplied with two or three levels of a highly available inorganic feed phosphate source which is considered as a reference standard (100% available). The test phosphates are then added to the same basal diet, often to provide the same inorganic P level. The availability of the test sources is then calculated by using a regression method. Because the availability (slope) of the test source is compared to the availability (slope) of the reference standard, availability values over 100% are possible. This leads to some additional confusion in the interpretation of obtained results. Values for bone mineral density and bone breaking strength may be additionally affected by the type of instrument used, procedures used to prepare the bones for testing, and physical and mechanical properties of bones (Orban et al., 1993). The results are also dependent on the response criterion and experimental techniques used. This all makes the data very variable between different researchers. Therefore, the information obtained from a RBA assay has limited value for a nutritionist formulating diets (Coon et al., 2002).

The performance and blood criteria seem to be not sensitive enough and therefore inappropriate for determining P availability, which was confirmed by the results presented in Chapter 4. It is also clear that blood criteria and performance may be affected by a much wider number of various factors in comparison to bone ash. Differences in performance can only be noted at large differences in bioavailability or large differences in mineral supply (Jongbloed and Kemme, 2002).

Therefore, the usage of either bone or blood or performance criteria as response criteria is not desirable for compiling feedstuff tables of P availability. In contrast, P retention and pc digestibility may yield quantitative values in P evaluation which can be also used in commercial feeding.

7.3. TIBIA P

Tibia P as a response criterion may play an important role in P evaluation. On the one hand, it is possible to determine only a relative value of P availability by using this criterion; on the other hand, this single bone could be representative of the total P content in the whole body. Results in Chapter 5 indicated that changes in TP may be suitable to predict the changes in whole body P WBP retention. Namely, the slope of linear regressions for both ages was identical (17.7) (Chapter 5), meaning that with each mg TP retention, WBP retention increased by 17.7 mg. If WBP retention was defined only on the basis of TP retention, the usage of the balance trials in P evaluation could be substituted by determining TP. A comparison between Δ WBP determined either on the basis of tibia (Chapter 4) or on the basis of pc digested P (Chapter 3) is presented in the Tables 27 and 28.

Table 27. ΔWBP determined on the basis of tibia and pc digested P in 3-wk-old broilers

Treatment	P content, g/kg feed DM	Tibia P, mg/bone	ΔTibia P[1], mg	Pc digested P[2], mg	ΔWBP[3], mg based on tibia P	ΔWBP[4], mg based on pc digestibility[5]
Basal diet	3.5	49.6		981		
MSP_a (0.08 % P)	4.3	61.3	11.7	1202	***207***	***221***
MSP_a (0.16 % P)	5.1	83.5	33.9	1808	***600***	***827***
MSP_a (0.24 % P)	5.9	102.3	52.7	1960	***933***	***979***
DCP_a (0.08 % P)	4.3	60.6	11.0	1098	***195***	***117***
DCP_a (0.16 % P)	5.1	72.3	22.7	1356	***402***	***375***
DCP_a (0.24 % P)	5.9	82.1	32.5	1557	***575***	***576***

[1] Calculated as the difference between tibia P of the inorganic phosphate supplemented diet and the tibia P of the basal diet

[2] Calculated by multiplying of P intake (mg) per average bird (pen) by pc digestibility value of the appropriate diet

[3] Calculated by multiplying of Δtibia P by the regression slope (17.7)

[4] Calculated by subtracting of pc digested P from the inorganic phosphate supplemented diet and the pc digested P of the basal diet

[5] pc digestibility was assumed to be equal retained P

Table 28. ΔWBP determined on the basis of tibia and pc digested P in 5-wk-old broilers

Treatment	P content, g/kg feed DM	Tibia P, mg/bone	ΔTibia P[1], mg	Pc digested P[2], mg	ΔWBP[3], mg based on tibia P	ΔWBP[4], mg based on pc digestibility[5]
Basal diet	3.5	273.9		2089		
MSP_a (0.08 % P)	4.3	331.4	58	2946	***1027***	***857***
MSP_a (0.16 % P)	5.1	378.8	105	3649	***1859***	***1560***
MSP_a (0.24 % P)	5.9	405.7	132	4139	***2336***	***2050***
DCP_a (0.08 % P)	4.3	310.1	36	2465	***637***	***376***
DCP_a (0.16 % P)	5.1	332.7	59	2693	***1044***	***604***
DCP_a (0.24 % P)	5.9	339.8	66	3002	***1168***	***913***

[1] Calculated as the difference between tibia P of the inorganic phosphate supplemented diet and the tibia P of the basal diet

[2] Calculated by multiplying of P intake (mg) per average bird (pen) by pc digestibility value of the appropriate diet

3 Calculated by multiplying of Δtibia P by the regression slope (17.7)

[4] Calculated by subtracting of pc digested P from the inorganic phosphate supplemented diet and the pc digested P of the basal diet

[5] pc digestibility was assumed to be equal retained P

It can be seen from Tables 27 and 28 that there was somehow not very good agreement between ΔWBP based on pc digestibility and ΔWBP based on TP in Period 1 and 2. Nevertheless, it must be emphasized that the calculations shown in Tables 27 and 28 are very rough. For instance, TP for a DCP_a (0.24%) supplemented diet was 82.1 mg/bone in 3-wk-old birds. This number, however, is an average of 32 values with a range from 63 to 97 mg/bone, which would lead to a large variation in ΔTP (mg) if calculated for each bone. The variation for this criterion was even much greater in 5-wk-old broilers (from 260 to 421 mg/bone). Thus, a variation between ΔWBP based on TP and ΔWBP based on pc digestibility

is comprehensible, which can be also seen in Tables 27 and 28. The slope of 17.7 in Chapter 5 was obtained using the individual values of TP and WBP.

Alternatively, Hurwitz (1964) determined net P utilization for inorganic phosphates by multiplying the regression slope of TP as a function of total P intake (mg/experimental period) by a factor of 1960. TP as a function of total P intake of broilers fed MSP_a and DCP_a during a 10-day experimental period in our study (Chapter 4) is shown in Figure 8.

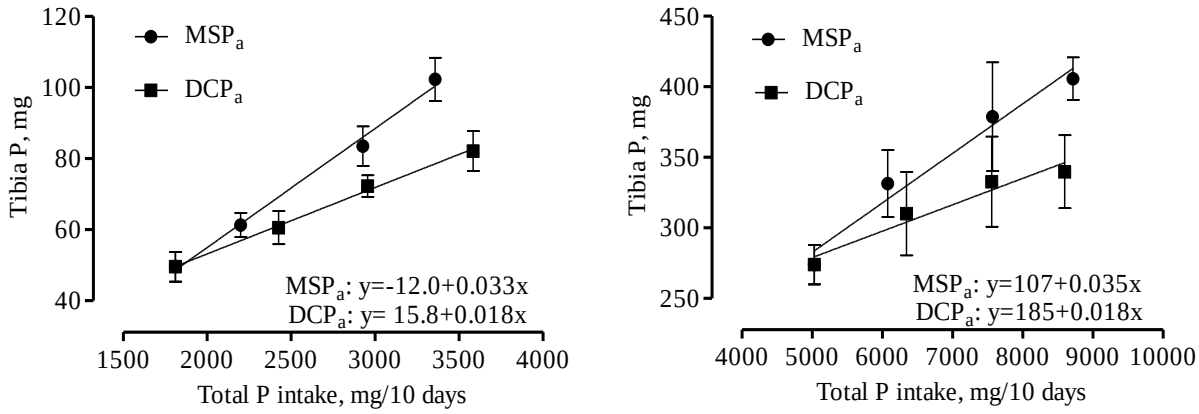

Figure 8. The linear relationship between total P intake during a 10-day experimental period and tibia P in 3- (left panel) and 5-wk-old (right panel) broilers

The net P utilization for MSP_a and DCP_a in Period 1 and 2 was calculated by multiplying the value of the corresponding regression slope by values of 2130 and 1980, respectively. These values (2130 and 1980) represent a WBP/TP ratio multiplied by 100 determined for 3- and 5-wk-old broilers (Chapter 5), respectively.

The net P utilization, pc digestibility and RBA of P from MSP_a and DCP_a in Period 1 and 2 are presented in the Table 29.

Table 29. Net P utilization (%), pc digestibility (%) (Chapter 3) and RBA[1] (%) of P from MSP_a and DCP_a in Period 1 and 2

	MSP_a	DCP_a
Period 1		
Net P utilization	70	38
Prececal digestibility	67	30
RBA	100	62
Period 2		
Net P utilization	69	36
Prececal digestibility	54	25
RBA	100	50

[1] Based on tibia ash weight (Chapter 4)

As one can see in Table 29, the net P utilization values calculated for MSP_a and DCP_a fit much better the pc digestibility than the respective RBA values, confirming that TP may present the amount of P retained/pc digested from a given supplement. There is also no difference in net P utilization between Period 1 and 2 for both phosphates, suggesting that there were no age-dependent (21 vs. 35 d of age) differences in the abilities of broilers to utilize P from either MSP_a or DCP_a, which is in agreement with what Rodehutscord et al. (2003) concluded for growing ducks.

Thus, TP may become a very useful tool in the P evaluation for broiler chicks, but further research is necessary. More precisely, differences between broiler strains or avian species need further investigation.

7.4. CONCLUSION

P pc digestibility and P retention seem to be the most appropriate criteria for a standardization of the P evaluation in broilers. Other factors such as the type and composition of diet are further essential factors to be standardized. The correlation analysis between tibia, tarsometatarsus, and toe bone ash weights indicated that the relative values based on these bone data were the same. However, the values obtained with bone data do not provide quantitative values of P availability which are needed by the poultry and feed industry. Thus, their use in P evaluation could be very limited. Blood P_i and performance data are not suitable as response criteria for assessing P availability. TP retention could be a good alternative to P retention determined in a balance trial. However, a further investigation is necessary. The use of a practical (corn-SBM-based) rather than a purified basal diet can be suggested in P evaluation because of its relevance to the poultry industry where phytate-containing diets are widely used.

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CHAPTER 8

SUMMARY

8. SUMMARY

Inorganic feed phosphates are an indispensable supplement for compounding poultry feed. The requirement of available P in broiler chicks cannot be covered only with plant ingredients as P in plant feedstuff is largely presented in form of phytate which is only partially available in avian species. Due to the increase in prices for feed phosphates and environmental concerns associated with excessive excretion of P by livestock, the knowledge about the availability of P from mineral sources has gained in importance during the last decade. However, there is still no standardized method available for assessing the P availability of inorganic feed phosphates. Without knowledge of the exact quantitative values of the P availability for different P sources, it is not possible to formulate adequate diets without the risk of deficiency or excess supplementation. There are various approaches which are used by different laboratories for the determination of P availability. The main problem is, however, that it is not clear how the differences between approaches affect the results. The development of a standardized method of P evaluation, which allows obtaining quantitative values for P availability, is the basis for optimizing the dietary P concentration in broiler diets.

The major objective of this thesis was to compare various methodological approaches that are used internationally to determine P availability in terms of their suitability. Therefore, firstly the P availability of two mineral phosphates was determined in 3- and 5-wk-old broilers based on data for P retention and prececal digestibility. The P availability of both mineral sources was calculated for both ages of birds by regression analyses for comparison of both response criteria. Secondly, the tibia bone ash and other bone criteria were determined. A comparison of these bone response criteria was then carried out by relating these data to measurements made on P retention. Thirdly, the suitability of tibia P retention for the estimation of the whole body P retention was investigated at both ages of birds. Variation in P retention of birds in these studies was additionally caused by the level and the source of P in the diet. In a fourth study, the effect of the basal diet composition on the availability of a feed phosphate was investigated based on quantitative P retention. A phytin-containing corn-SBM-based as well as a purified basal diet was used. Moreover, the impact of the inorganic phosphate level on the IP_6 hydrolysis of the corn-SBM-based diet was assessed on the basis of excreta collection.

In the first study, a corn-SBM-based basal diet was used (0.35% P on dry matter basis). MSP_a or DCP_a was supplemented to increment the P concentration by 0.08%, 0.16%, and 0.24%. Two balance trials (n=8 birds per diet) and two digestibility trials (n=8 pens with 10 birds per diet) were conducted (8 treatments per diet). In 3-wk-old broilers, P retention for MSP_a was 70% and significantly higher ($P < 0.001$) than for DCP_a (29%), as calculated by linear regression analysis. Values determined for P pc digestibility at the same age were very similar (67% for MSP_a and 30% for DCP_a; $P < 0.001$). In 5-wk-old broilers, P retention was 63% (MSP_a) and 29% (DCP_a) ($P < 0.001$), and pc digestibility was 54% (MSP_a) and 25% (DCP_a) ($P = 0.002$). In conclusion, in 3-wk-old broilers results obtained with both approaches were the same. In 5-wk-old broilers, the ranking of the two P sources was the same for both approaches. Values differed not greatly between the two age periods.

The second study was linked to the first one, and the experimental design was the same. The study comprised two periods with birds of different ages, but from the same hatch. The response criteria evaluated were tibia, tarsometatarsus, toe ash, and P, as well as the Quantitative Computed Tomography measurements of tibiae, blood P_i concentration, and body weight gain. Responses were evaluated and compared based on linear regression analysis. In general, MSP_a had a greater slope than DCP_a for all criteria studied. For the different bones, the ratio of slopes was very similar based on the amount of ash in both periods. Foot ash was proved to be as sensitive as tibia ash in both periods. Blood serum P_i and body weight gain were not sufficiently sensitive criteria for P evaluation. We concluded that the ranking of both mineral P sources based on bone criteria differed from the ranking that was based on P retention or pc digestibility.

The third study was also linked to the first one. Thus, the experimental design was the same. On days 21 and 35, two chicks per treatment were randomly chosen. Contents of P and Ca were determined in tibiae-free bodies and tibiae. The whole body P to tibia P ratio was 21.3±1.3 at d 21 and 19.8±1.1 at d 35 of age. The slope of linear regressions between the tibia P and the whole body P for both ages was identical (17.7). Results indicated that changes in tibia P may be suitable to predict changes in whole body P retention.

In the last experiment, a phytin-containing as well as a purified basal diet, both containing 1.8 g available P per kg feed dry matter, was supplemented with MSP_a to increment the P concentration by 0.05%, 0.1%, and 0.15%. A retention trial with excreta collection from d 20-24 was conducted (n=7 birds per diet). The level of P did not significantly affect the total P retention either of the corn-SBM-based or of the purified basal diet ($P > 0.05$). However, increasing the P level significantly reduced ($P = 0.015$) the IP_6

hydrolysis for the corn-SBM-based diets. Percentage P retention for MSP_a was calculated by linear regression analysis. P retention for MSP_a was 50% for the corn-SBM-based diet and 51% for the purified diet. We concluded that there was no difference in P retention from MSP_a between corn-SBM-based and purified diets.

It can be concluded from the results of the present thesis that both retention and pc digestibility can be used for evaluating mineral P sources in broilers based on a regression approach. The ranking of mineral P sources based on bone criteria differed from the ranking that was based on P retention or pc digestibility. There was no difference in P retention from MSP_a between corn-SBM-based and purified based diets, but a significant effect of the P-level on the IP_6 hydrolysis in corn-SBM-based basal diets was found.

CHAPTER 9

ZUSAMMENFASSUNG

9. ZUSAMMENFASSUNG

Anorganische Phosphate stellen eine unverzichtbare P-Quelle im Geflügelfutter dar. Der Bedarf an verwertbarem Phosphor (P) für den Broiler kann nicht allein über pflanzliche Rationskomponenten gedeckt werden, da pflanzlicher P zumeist in Form von Phytat vorliegt, welches nur teilweise durch das Geflügel verwertbar ist. Bedingt durch den Anstieg der Preise für Futterphosphate und ökologische Bedenken, die mit der übermäßigen P-Ausscheidung durch landwirtschaftliche Nutztiere verbunden sind, haben Erkenntnisse zur Verwertbarkeit des P aus mineralischen Quellen während des letzten Jahrzehntes zunehmend an Bedeutung gewonnen. Allerdings gibt es noch keine standardisierte Methode zur Beurteilung der P-Verwertbarkeit aus anorganischen Futterphosphaten. Ohne genaue Kenntnis quantitativer Werte zur P-Verwertbarkeit verschiedener Futterphosphate ist es allerdings nicht möglich, Geflügelrationen ohne das Risiko einer Unter- oder Übersupplementierung zu formulieren. In verschiedenen Laboren gibt es international unterschiedliche Ansätze zur Bestimmung der P-Verwertbarkeit. Das Hauptproblem hierbei besteht darin, dass nicht klar ist, inwieweit die unterschiedlichen Herangehensweisen die Vergleichbarkeit der Ergebnisse beeinflussen. Die Entwicklung einer standardisierten Methode für die P-Bewertung, die eine Ermittlung quantitativer Werte für die P-Verwertbarkeit ermöglicht, ist aber Grundlage für die Optimierung des P-Gehaltes im Futter für Broiler.

Das Hauptziel dieser Arbeit war daher, verschiedene methodische Ansätze, die international zur Ermittlung der P-Verwertbarkeit zum Einsatz kommen, hinsichtlich ihrer Eignung zu vergleichen. Hierfür wurde zunächst die P-Verwertbarkeit zweier mineralischer Phosphate in 3- und 5-Wochen alten Broilern, basierend auf P-Retention und praecaecaler (pc) Verdaulichkeit, bestimmt. Die P-Verwertbarkeit der beiden mineralischen Quellen wurde für beide Altersgruppen durch lineare Regressionsanalyse berechnet und diese dann für beide Kriterien miteinander verglichen. Zweitens, wurden die Tibia-Knochenasche und andere Knochen-Kriterien bestimmt. Anschließend wurde ein Vergleich der Knochen-Kriterien basierend auf den Ergebnissen zur P- Retention durchgeführt. Drittens, wurde die Eignung der Tibia-P (TP)-Retention zur Schätzung der P-Retention im Ganzkörpers (WBP) für beide Altersabschnitte untersucht. Eine weitere Variation in der P-Retention der Tiere wurde in diesen Studien durch unterschiedliche P-Versorgung und variierende P-Quellen im Futter erreicht. In einer vierten Studie wurde der Einfluss unterschdiedlich zusammengesetzter Basalrationen auf die P-Verwertbarkeit eines Futterphosphates anhand der quantitativen P-

Retention untersucht. Eine Phytat-haltige, auf Mais und Sojaextraktionsschrot (SBM) basierende, und eine synthetische Basalration sind hierfür verwendet worden. Darüber hinaus wurde der Einfluss des Niveaus der anorganischen Phosphatzulage auf die Inositol-6-Phosphat (IP_6)-Hydrolyse der Mais-SBM-Diät anhand der Exkremente beurteilt.

In der ersten Studie kam eine auf Mais und SBM basierende Basalration zum Einsatz (0,35% P auf Trockensubstanz (TM)-Basis). Wasserfreies Mononatriumphosphat (MSP_a) oder wasserfreies Calciumhydrogenphosphat (DCP_a) wurde stufenweise zugelegt, um die P-Konzentration in der Ration um 0,08, 0,16 und 0,24% zu erhöhen. Es wurden sowohl zwei Bilanzversuche zur Ermittlung der P-Retention als auch zwei Versuche zur Bestimmung der pc Verdaulichkeit durchgeführt (8 Behandlungen pro Diät). Wie durch lineare Regression berechnet, betrug die P-Retention aus MSP_a in den 3 Wochen alten Broilern 70% und war damit signifikant höher ($P < 0,001$) als für das DCP_a (29%). Die Werte für die pc P-Verdaulichkeit in diesem Altersabschnitt lagen in einem ähnlichen Bereich (67% für MSP_a und 30% für DCP_a, $P < 0,001$). Für die 5 Wochen alten Tiere wurde eine P-Retention von 63% (MSP_a) bzw. 29% (DCP_a) ($P < 0,001$) und eine pc P-Verdaulichkeit von 54% (MSP_a) bzw. 25% (DCP_a) ($P = 0,002$) ermittelt. Schlussfolgernd kann festgehalten werden, dass für die 3 Wochen alten Broiler beide Parameter die gleichen Ergebnisse lieferten. In den 5 Wochen alten Tieren war die Rangierung beider P-Quellen gleich, wenn die Ergebnisse für beide Kriterien miteinander verglichen wurden. Zwischen beiden Altersabschnitten unterschieden sich die Werte nur unwesentlich.

Die zweite Studie war eng mit der ersten verknüpft. So war das experimentelle Design gleich. Die Studie umfasste ebenfalls zwei Perioden mit Broilern unterschiedlichen Alters, die aber dem gleichen Schlupf und Fütterungsversuch entstammten. Die zu bewertenden Responskriterien waren die Asche- und P-Gehalte in den Tibiaknochen, dem Tarsometatarsus und den Zehen. Außerdem wurden verschiedene quantitative Messungen an der Tibia mittels Computertomographen durchgeführt. Darüber hinaus wurden die P_i-Konzentration im Blutserum sowie die Körpermassezunahme der Tiere erhoben. Die lineare Regressionsanalyse war auch hier Grundlage für die Auswertung und den Vergleich der einzelnen Responskriterien. Für jedes der untersuchten Kriterien zeigte sich im Falle des MSP_a eine größere Steigung als für das DCP_a. Bezogen auf die Aschemenge in den verschiedenen Knochen konnte in beiden Altersabschnitten ein ähnliches Verhältnis der Steigungen festgestellt werden. In beiden Perioden erwies sich die Fuß-Asche als ebenso empfindlich wie die Tibia-Asche. Die P_i-Konzentration im Blutserum sowie die Körpermassezunahme stellten sich als nicht hinreichend empfindliche Kriterien für die P-Bewertung heraus. Hieraus ließ

sich schließen, dass die Rangierung der beiden mineralischen P-Quellen basierend auf den Knochenkriterien anders ausfiel als über die P-Retention oder pc Verdaulichkeit ermittelt.

Auch die dritte Studie war eng mit der ersten verknüpft. An den Tagen 21 und 35 wurden zwei Tiere pro Behandlung nach dem Zufallsprinzip ausgewählt, um den Gehalt an P und Ca im Tibia-freien Körper und den Tibiae zu bestimmen. Das Verhältnis von WBP/Tibia-P betrug 21,3 ± 1,3 bei den 21 Tage alten und 19,8 ± 1,1 bei den 35 Tage alten Broilern. Die Steigung der linearen Regression zwischen dem Tibia-P-Gehalt und dem WBP war identisch (17,7) für beide Altersabschnitte. Diese Ergebnisse sind ein deutlicher Hinweis, dass die Veränderungen im P-Gehalt der Tibia genutzt werden können, um Veränderungen in der P-Retention des Ganzkörpers abzuschätzen.

Im letzten Experiment wurden sowohl eine Phytin-haltige als auch eine synthetische Basalration (1,8 g verwertbarer P je kg Futter TM) mit MSP_a ergänzt, um P-Konzentrationen von 0,05, 0,1 und 0,15% in den Rationen zu erreichen. Es wurde ein Retentionsversuch auf Basis von Exkrementensammlungen zwischen dem 20. und 24. Lebenstag durchgeführt (n = 7 Tiere pro Diät). Die Höhe der P-Versorgung hatte weder im Falle der Mais-SBM-basierten noch bei Verwendung der synthetischen Basalration einen signifikanten Einfluss auf die Gesamt-Retention des P durch die Tiere ($P > 0,05$). Die Erhöhung des P-Niveaus reduzierte aber signifikant ($P = 0,015$) die IP_6-Hydrolyse aus den Mais-SBM-basierten Mischungen. Die prozentuale P-Retention aus dem MSP_a wurde durch lineare Regressionsanalyse ermittelt. Die P-Retention für MSP_a betrug hier 50% für die Mais-SBM-basierten Diäten und 51% für die synthetischen Diäten. Folglich konnte für diese beiden Basaldiättypen kein Unterschied in der P-Retention aus dem MSP_a festgestellt werden.

Aus den Ergebnissen der vorliegenden Arbeit läßt sich schlussfolgern, dass sowohl die P-Retention als auch die pc P-Verdaulichkeit geeignete Kriterien sind, um eine Bewertung mineralischer P-Quellen für das Masthuhn auf Basis linearer Regressionen vorzunehmen. Die Rangierung der mineralischen P-Quellen basierend auf den Knochenkriterien unterschied sich von dem Ranking, das auf P-Retention oder pc Verdaulichkeit basierte. Es gab keinen Unterschied in der P-Retention für MSP_a zwischen Mais-SBM-basierten und synthetischen Diäten, allerdings war ein signifikanter Effekt des P-Supplementationsniveaus auf die IP_6-Hydrolyse aus Mais-SBM-basierten Diäten zu verzeichnen. Für die Entwicklung eines Standard-Protokolls zur Ermittlung des verwertbaren P beim Geflügel wurden hiermit Beiträge geliefert und Impulse gegeben.

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Erklärung

Die Dissertation wurde von mir selbstständig angefertigt. Es wurden nur die angegebenen Quellen als Hilfsmittel verwendet. Wörtlich oder inhaltlich übernommene Stellen wurden als solche gekennzeichnet.

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