Genetic parameters for bone ash and phosphorus utilization in an F_2 cross of Japanese quail

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ABSTRACT The main objective of this study was to perform quantitative genetic analyses of tibia and foot ash traits, which might serve as proxy traits to improve phosphorus utilization (**PU**) in a breeding program. Additionally, data for ash concentration in tibia and foot were compared with data for total amount of ash. Heritabilities for bone ash traits and genetic and phenotypic correlations between bone ash traits and PU were estimated. A total of 887 F_2 birds, established from 2 Japanese quail lines divergently selected on social reinstatement behavior, were provided a P deficient diet. In a metabolic study, feed consumption was measured and total excreta collected for each bird separately. Afterwards, birds were euthanized, the bones obtained and incinerated. Bone ash data showed a heritability of 0.230 (amount of tibia ash) to 0.342 (amount of foot ash), which was higher than estimated for PU, P retention, calcium utilization (0.120–0.174), and performance traits (0.088–0.114). The strongest genetic and phenotypic correlations between PU and bone ash traits were detected for the amount of foot ash with 0.549 and 0.527, respectively. Genetic and phenotypic correlations were stronger between PU and ash amount than between PU and ash percentage, irrespective of bone. Therefore, ash amount was considered a better trait than ash percentage to reflect PU. Strong genetic and phenotypic correlations were detected between the amount of foot and tibia ash (0.887 and 0.901, respectively). Phenotypic and genetic correlations between ash amount and PU or calcium utilization were almost identical, irrespective of bone. Foot ash is as suitable as tibia ash, but easier to determine. Bone ash data, especially the amount of foot ash, seem to be suitable indirect selection criteria for P efficiency breeding.

Key words: phosphorus utilization, heritability, tibia ash, foot ash, indirect selection trait

2019 Poultry Science 98:4369–4372
http://dx.doi.org/10.3382/ps/pez398

INTRODUCTION

Phosphorus (**P**) is a mineral of crucial importance for all living organisms. It is needed for bone mineralization, energy metabolism, and other metabolic processes in the body. Due to globally limited availability of rock P resources and environmental impact of P contained in excreta, but also for economic reasons, it is desirable to minimize mineral P supplementation of poultry feed. *Myo*-inositol 1,2,3,4,5,6-hexakis(dihydrogen phosphate) ($InsP_6$), the main storage form of P in plant seeds, is only partially available for non-ruminants (Eeckhout and Paepe, 1994; Rodehutscord et al., 2016). For this reason, poultry diets are commonly supplemented with mineral P, phytase or both.

The potential of broiler chickens for gastrointestinal $InsP_6$ degradation is high when diets have a low P concentration, indicating an endogenous phytase activity originating from the epithelial tissue or gut microbiota (Rodehutscord and Rosenfelder, 2016). Endogenous phytase activity might be affected by the genome of the animal. Japanese quail are important model organisms in poultry studies (Minvielle, 2004; Rodehutscord and Dieckmann, 2005) with the advantages of requiring less space, having a shorter generation interval and growing faster compared to other poultry species. A moderate heritability of phosphorus utilization (**PU**) was estimated, with values of 0.136 for Japanese quail (Beck et al., 2016) and 0.10 for broiler chickens (Zhang et al., 2003). However, even in quail, PU is difficult to determine under practical conditions and with a large number of animals, because it needs analysis of ileal digesta or sampling of excreta. Thus, an alternative trait that could be used for indirect selection for improved P efficiency would be beneficial. Possible traits are tibia or foot ash; both are easier to determine than the target trait PU. Tibia ash and foot ash are often used as response criteria in nutritional studies of P bioavailability in poultry (Yan et al., 2005; Shastak et al., 2012a; Shastak and Rodehutscord, 2013).

Received January 18, 2019.
Accepted June 13, 2019.

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However, heritability of tibia and foot ash as well as correlations with PU are rarely known to date. Hence, the main objective of this study was to perform quantitative genetic analyses of bone ash traits and to study their interrelationship with PU and various performance traits. Additionally, the use of tibia and foot ash and the use of relative or absolute ash data were compared as criteria of bone mineralization and in genetic analysis.

MATERIALS AND METHODS

Experimental Design

The experiment was conducted in accordance with the German Animal Welfare Legislation approved by the Animal Welfare Commissioner of the University. An F_2 cross of Japanese quail (Coturnix japonica) was used. The experimental design and procedures are described in detail by Beck et al. (2016) and the present work is an extension of that experiment. In brief, a F_2-design using two Japanese quail lines divergently selected on social reinstatement behavior was established. Selection of these founder-lines took place at INRA, Nouzilly (France) and was described by Mills and Faure (1991). In their 6th wk of life, 12 males of the F_0-generation from line A (B) were mated to 12 females from line B (A). A total of 17 roosters and 34 hens were randomly selected from the F_1-birds in their 6th week of life. One rooster was paired with two hens to generate 920 F_2-animals, whereof 887 were used for analyses following a check of plausibility of data and removal of outliers (Beck et al. 2016). The quails were generated in 12 consecutive hatches with 60–100 individuals each.

In their first 5 d of life, the F_2-animals were fed a commercial starter diet. From days 6 to 15, a low-P diet based on corn, corn starch and soybean meal was provided for ad libitum consumption. The diet did not contain a mineral P supplement or phytase. The low P content (4.0 g/kg DM) was chosen to let the birds express their full genetic potential of PU, as recommended by WPSA (2013). After 7 d of raising in groups on floor pens, F_2-birds were transferred to metabolic cages, where they were kept individually but with visual contact to neighbors. The first 2 d in these cages were for adaptation, followed by a 5-d period for phenotyping, where individual feed consumption was measured and total excreta were collected. On day 15 of age, the experiment was terminated.

Trait Measurements

Quails were weighed on days 10 and d 15 and bodyweight gain (**BWG**) was calculated as the difference between the 2 weights. Feed per gain ratio was calculated as feed intake per BWG in this period. Analyses of P and Ca in the diet and sampled excreta were done according to Shastak et al. (2012b), using an inductively coupled plasma optical emission spectrometer. For calculation of PU and calcium utilization (**CaU**), the difference between total intake and excretion during the phenotyping period for each individual was used (Beck et al., 2016).

Quails were slaughtered at the end of the phenotyping period, dissected, and tissues immediately frozen. The right tibiotarsus (tibia) of each animal was cleaned from adhering tissues, fibula bones, and cartilage caps after defrosting. The right foot of each animal was taken including skin, claws, and all tissues below the articulatio intertarsalis. Subsequently, tibiae and feet were rinsed with distilled water and dried with a lint-free paper towel. Dry matter content of tibiae and feet was determined at 103°C for 24 h in a compartment oven (VL 115, VWR International GmbH, Darmstadt, Germany). Ash content was determined after 16 h of incineration at 550°C in a muffle furnace (L 40/11/B170, Nabertherm GmbH, Lilienthal, Germany). After placement of the bones in the furnace, they were heated up for a period of 7 h and cooled down for a period of 5 h. The total amount of ash present in tibia and foot (**TA mg** and **FA mg**) as well as ash concentrations in the dry matter of bones (**TA %** and **FA %**) were considered in analyses.

Statistical Analysis

Data were analyzed using the following linear mixed model with the ASReml software (Gilmour et al., 2006):

$$y = Xb + Z_h h + Z_a a + e$$

where y = vector of observations, b = vector of the fixed overall mean, h = vector with random hatch effects, a = vector with the random additive-genetic effects of the individuals, X, Z_h and Z_a = corresponding design matrixes, and e = residual term.

The covariance structure of the random animal effect was $var\ (a) = A * \sigma_a^2$, where A = pedigree-based numerator relationship matrix and σ_a^2 = additive genetic variance. The variance of the random hatch effect was $var\ (h) = I * \sigma_h^2$, where I = identity matrix and σ_h^2 = hatch variance. The variance structure of the random residual effect was $var\ (e) = I * \sigma_e^2$, where σ_e^2 = residual variance. Heritabilities of the traits were estimated with univariate analyses. For estimation of genetic and phenotypic correlations, pairwise bivariate analyses using the same models were conducted.

RESULTS AND DISCUSSION

The genetic parameter estimates are shown in Table 1. In general, the standard errors of parameters were small for the results from the univariate analysis, but larger for bivariate analysis. This implies that the structure and size of the experiment is sufficient for the heritability estimation, but the genetic correlation

Table 1. Estimated phenotypic correlations (above the diagonal), genetic correlations (below the diagonal), and trait heritability (on the diagonal in bold), n = 887.

	TA mg	TA %	FA mg	FA %	PU	PR	CaU	BWG	FI	F:G
TA mg	**0.230**[a]	0.567*	0.901	0.510	0.511	0.753	0.646	0.584	0.740	−0.068
TA %	0.593[b]	**0.234**[a]	0.536	0.688	0.348	0.359	0.561	0.149	0.275	0.135
FA mg	0.887[a]	0.600[b]	**0.342**[a]	0.590	0.527	0.752	0.662	0.557	0.727	−0.056
FA %	0.544[b]	0.593[b]	0.687[b]	**0.310**[a]	0.268	0.293	0.530	0.009	0.211	0.277
PU	0.495[b]	0.462[c]	0.549[b]	0.464[c]	**0.134**[a]	0.789	0.839	0.599	0.552	−0.446
PR	0.866[a]	0.474[c]	0.943[a]	0.507[b]	0.705[b]	**0.120**[a]	0.694	0.837	0.932	−0.312
CaU	0.688[b]	0.619[b]	0.716[b]	0.599[b]	0.926[a]	0.776[b]	**0.174**[a]	0.370	0.478	−0.099
BWG	0.742[a]	0.177[c]	0.735[a]	0.098[c]	0.636[b]	0.905[a]	0.525[b]	**0.088**[a]	0.857	−0.652
FI	0.737[a]	0.282[c]	0.896[a]	0.307[b]	0.432[c]	0.936[a]	0.494[b]	0.869[a]	**0.114**[a]	−0.240
F:G	0.056[c]	0.198[c]	0.179[c]	0.374[c]	−0.450[c]	0.043[c]	−0.195[c]	−0.217[c]	0.238[c]	**0.110**[a]

[a]Standard error ≤ 0.10.
[b]Standard error between 0.11 and 0.20.
[c]Standard error ≥ 0.21.
*All phenotypic correlations have standard errors between 0.005 and 0.042.
Abbreviations: TA, tibia ash; FA, foot ash; PU, phosphorus utilization; PR, phosphorus retention; CaU, calcium utilization; BWG, bodyweight gain; FI, feed intake; F:G, feed per gain.

estimates have to be interpreted with some caution due to the larger standard errors.

Heritability of Bone Ash Traits

A medium heritability was detected for bone ash data with values from 0.230 (TA mg) to 0.342 (FA mg, Table 1). These estimates of heritability were higher than those estimated for PU, phosphorus retention (**PR**), and CaU (0.120–0.174) or performance traits (0.088–0.114). The heritabilities for FA mg (0.342) and FA % (0.310) were higher than those for TA mg (0.230) and TA % (0.234). Heritabilities of all bone ash traits in the present study are within the range estimated from other authors for TA in broiler chickens. For instance, heritability for TA % was 0.08 in an unselected broiler control population after feeding a diet with 7.2 g total P/kg (González-Cerón et al., 2015). Verdal et al. (2013) reported higher heritabilities of 0.41 for TA mg and 0.52 for TA % in broilers divergently selected either for high or low digestive efficiency feeding a diet with 6.6 g total P/kg.

Correlations between Bone Ash and other Traits

Standard errors of all estimated genetic correlations were large and thus have to be interpreted with some caution. The strongest genetic and phenotypic correlations between PU and bone ash traits were detected for FA mg with 0.549 and 0.527, respectively (Table 1). PU was on a high level with a mean value of 71.4%, but varied widely with a range of 21.5–87.4% (Table 2), which is partially due to different additive-genetic effects of the individuals, as can be deduced from the heritability of PU (Table 1, Beck et al. 2016). For PR, the strongest genetic correlation was detected with FA mg (0.943), and the strongest phenotypic correlation was with TA mg (0.753) and FA mg (0.752).

Table 2. Abbreviations (Abbr), mean, minimum (Min), maximum (Max), standard deviation (SD), and coefficient of variation (CV) of the observed traits of the Japanese quail F_2-animals, n = 887.

Trait	Unit	Abbr	Mean	Min	Max	SD	CV (%)
Tibia ash[1]	mg	TA mg	45.8	19.2	83.5	9.24	10
Tibia ash[1]	%	TA %	45.3	35.5	55.7	2.59	3
Foot ash[1]	mg	FA mg	44.8	19.6	83.6	8.23	11
Foot ash[1]	%	FA %	17.3	12.1	21.9	1.40	5
P utilization[2]	%	PU	71.4	21.5	87.4	8.00	4
P retention[2]	g DM	PR	0.11	0.01	0.18	0.03	8
Ca utilization[2]	%	CaU	60.6	19.4	84.3	10.0	7
Bodyweight gain[2]	g	BWG	24.5	5.8	37.9	5.04	6
Feed intake[2]	g	FI	42.7	16.1	62.4	7.13	6
Feed per gain[2]	g/g	F:G	1.78	1.21	3.92	0.30	6

[1]d15.
[2]d10–15.

The phenotypic and genetic correlations between all bone ash traits and PR were stronger than between bone ash traits and PU. PR represents the total amount of dietary P retained in the animal, and the by far highest proportion of it is retained in the bones. Therefore, bone ash has been considered a good indicator for bone mineralization and relative bioavailability of P in poultry (Shastak et al., 2012a). However, because PR is not independent from feed intake and feed intake cannot easily be determined in a large population, PU can be considered as the more suitable trait for P efficiency breeding.

Genetic and phenotypic correlations were stronger between bone ash data and CaU than between bone ash data and PU. This is likely caused by the higher amount of Ca stored in the bone compared to P. Suchý et al. (2009) reported Ca in TA to be more than twice as high as P with a P: Ca ratio of 1:2.18 in 40-d old broilers.

Comparison of Bone Ash Amount and Percentage

Genetic and phenotypic correlations were stronger between PU or PR and ash amount than ash percentage for FA and TA. Therefore, ash amount seems to be

a better trait to reflect PU and PR than ash percentage. This is in agreement with Shastak et al. (2012a) and Li et al. (2015), who reported a better reflection of bone mineralization by the use of ash amount than ash percentage. The amount of ash considers the size of the bone and therefore overall mineralization, while the relative value of ash percentage is less sensitive. Ash percentage is not a direct reflection of the Ca and P deposited in bone, since it removes bone weight differences observed between treatments (Li et al., 2015). However, most studies investigating bone mineralization have used relative values.

Comparison of Tibia and Foot Ash

The tibia is the most commonly used bone for determination of bone mineralization. However, other bones like the femur or a toe also have been used. Some authors concluded that foot ash can be used as an alternative to tibia ash in broiler chickens and recommended the use of foot ash for assessing bone mineralization (Yan et al., 2005; Garcia and Dale, 2006; Shastak et al., 2012a; Malloy et al., 2017). However, genetic analyses for foot ash were not conducted before.

In the present study, mean, minimum, and maximum values were very similar for TA mg and FA mg (Table 2) and strong genetic and phenotypic correlation existed between these 2 traits (0.887 and 0.901, respectively, Table 1). Phenotypic correlations between ash amount and PU (0.511 and 0.527 for tibia and foot, respectively), PR (0.753 and 0.752 for tibia and foot, respectively), or CaU (0.646 and 0.662 for tibia and foot, respectively) were almost identical for tibia and foot. Genetic correlations were marginally lower for TA mg than for FA mg. For ash percentage, TA showed stronger phenotypic correlations with PU, PR, and CaU than FA, genotypic correlations were almost identical. Determination of foot ash is less laborious than tibia ash. While the tibia has to be carefully defleshed before incineration, the whole food can be easily cut at the articulatio intertarsalis and does not need to be processed otherwise. Results from the present study showed that foot ash can be used as an alternative to tibia ash in determination of relative P bioavailability and genetic analysis.

In conclusion, bone ash data, especially the amount of FA, are suitable indirect selection criteria for P efficiency breeding. Bone ash traits show strong correlations with PU and PR, are easier to determine in a large number of animals, and have a higher heritability. The total amount of bone ash is a better indicator for PU and PR than bone ash concentration, and total PR values are better reflected than relative PU values. Foot ash is as suitable as tibia ash and less laborious to determine. Results of the present study should be confirmed in poultry breeding populations and using larger data sets in order to obtain smaller standard errors of the correlation coefficient estimates.

REFERENCES

Beck, P., H.-P. Piepho, M. Rodehutscord, and J. Bennewitz. 2016. Inferring relationships between Phosphorus utilization, feed per gain, and bodyweight gain in an F_2 cross of Japanese quail using recursive models. Poult. Sci. 95:764–773.

de Verdal, H., A. Narcy, D. Bastianelli, N. Même, S. Urvoix, A. Collin, E. Le Bihan-Duval, and S. Mignon-Grasteau. 2013. Genetic variability of metabolic characteristics in chickens selected for their ability to digest wheat. J. Anim. Sci. 91:2605–2615.

Eeckhout, W., and M. de Paepe. 1994. Total phosphorus, phytate-phosphorus and phytase activity in plant feedstuffs. Anim. Feed Sci. Technol. 47:19–29.

Garcia, A. R., and N. M. Dale. 2006. Foot ash as a means of quantifying bone mineralization in chicks. J. Appl. Poult. Res. 15:103–109.

Gilmour, A. R., B. J. Gogel, B. R. Cullis, and R. Thompson. 2006. ASReml user Guide Release 2.0. VSN International Ltd, Hemel Hempstead, UK.

González-Cerón, F., R. Rekaya, and S. E. Aggrey. 2015. Genetic analysis of bone quality traits and growth in a random mating broiler population. Poult. Sci. 94:883–889.

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

Malloy, M. N., A. G. Stephens, M. E. Freeman, M. K. Jones, J. M. Faser, N. M. Dale, and A. J. Davis. 2017. Foot ash can replace tibia ash as a quantification method for bone mineralization in broilers at 21 and 42 days of age. J. Appl. Poult. Res. 26:175–182.

Mills, A. D., and J.-M. Faure. 1991. Divergent selection for duration of tonic immobility and social reinstatement behavior in Japanese quail (Coturnix coturnix japonica) chicks. J. Comp. Psychol. 105:25–38.

Minvielle, F. 2004. The future of Japanese quail for research and production. World's Poult. Sci. J. 60:500–507.

Rodehutscord, M., and A. Dieckmann. 2005. Comparative studies with three-week-old chickens, turkeys, ducks, and quails on the response in phosphorus utilization to a supplementation of monobasic calcium phosphate. Poult. Sci. 84:1252–1260.

Rodehutscord, M., and P. Rosenfelder. 2016. Update on phytate degradation pattern in the gastrointestinal tract of pigs and broiler chickens. Pages 15–28. In Phytate Destruction – Consequences for Precision Animal Nutrition, C. L. Walk, I. Kühn, H. H. Stein, M. T. Kidd, and M. Rodehutscord, (eds.) Wageningen Academic Publishers, Wageningen - Netherlands.

Rodehutscord, M., C. Rückert, H. P. Maurer, H. Schenkel, W. Schipprack, K. E. Bach Knudsen, M. Schollenberger, M. Laux, M. Eklund, W. Siegert, and R. Mosenthin. 2016. Variation in chemical composition and physical characteristics of cereal grains from different genotypes. Arch. Anim. Nutr. 70:87–107.

Shastak, Y., and M. Rodehutscord. 2013. Determination and estimation of phosphorus availability in growing poultry and their historical development. World's Poult. Sci. J. 69:569–586.

Shastak, Y., M. Witzig, K. Hartung, W. Bessei, and M. Rodehutscord. 2012. Comparison and evaluation of bone measurements for the assessment of mineral phosphorus sources in broilers. Poult. Sci. 91:2210–2220.

Shastak, Y., M. Witzig, K. Hartung, and M. Rodehutscord. 2012. Comparison of retention and prececal digestibility measurements in evaluating mineral phosphorus sources in broilers. Poult. Sci. 91:2201–2209.

Suchý, P., E. Straková, I. Herzig, L. Steinhauser, G. Králik, and D. Zapletal. 2009. Chemical composition of bone tissue in broiler chickens intended for slaughter. Czech J. Anim. Sci. 54:324–330.

WPSA. 2013. Determination of phosphorus availability in poultry. World's Poult. Sci. J. 69:687–698.

Yan, F., C. A. Keen, K. Y. Zhang, and P. W. Waldroup. 2005. Comparison of methods to evaluate bone mineralization. J. Appl. Poult. Res. 14:492–498.

Zhang, W., S. E. Aggrey, G. M. Pesti, H. M. Edwards, and R. I. Bakalli. 2003. Genetics of phytate phosphorus bioavailability: Heritability and genetic correlations with growth and feed utilization traits in a randombred chicken population. Poult. Sci. 82:1075–1079.