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Determination of calcium and phosphorus utilization in various hatchery by-products for broiler chickens

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Abstract

The objective of the current study was to determine calcium (Ca) and phosphorus (P) utilization in 4 different hatchery by-products (HBPs) for broiler chickens. The four different HBPs included infertile eggs (IFE), unhatched eggs (UHE), low grade and dead chicks (LDC), and a mixture (MIX) of 55% IFE, 10% UHE, and 10% LDC with 25% hatched eggshells. A total of sixty four 50-day-old Ross 308 broiler chickens were randomly allotted to 1 of 4 dietary treatments with 8 replicates per treatment. Two birds were placed together in one metabolic cage. Additional 16 birds were used to measure the endogenous losses of Ca and P. A force-feeding procedure (i.e., crop intubation) was used to measure the apparent and true total tract retention (ATTR and TTTR, respectively) of Ca and P in the 4 HBPs. The results showed that the TTTR of Ca in the UHE was less ($p < 0.05$) than that in the IFE, LDC, and MIX. The amounts of available Ca in the MIX were greater ($p < 0.05$) than those in the IFE and UHE, which were greater ($p < 0.05$) than those in the LDC. The TTTR of P was not different among the 4 HBPs. However, the amounts of available P in the LDC were greater ($p < 0.05$) than those in the IFE and UHE, which were greater ($p < 0.05$) than those in the MIX. In conclusion, HBPs contain high amounts of available Ca and P because of high concentrations of total Ca and P with a high utilization rate. Therefore, the use of HBPs in broiler diets can reduce costs on Ca and P supplements.

Keywords: broiler chicken, calcium, hatchery by-product, phosphorus

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Introduction

The rapid growth of poultry industry leads to a large production of poultry by-products (Das et al., 2002). Hatchery by-products (HBPs) are one of the poultry by-products. HBPs include all kinds of by-products produced in poultry hatcheries during the period of hatching and after hatching. Typically, HBPs can be classified into infertile eggs (IFE), unhatched eggs (UHE), low grade and dead chicks (LDC), or hatched eggshells (HE) (Hamm and Whitehead, 1982; Rasool et al., 1999; Shahriar et al.,

2008), although these HBPs are generally collected in a mixture (MIX) of IFE, UHE, LDC, and HE at the end of hatching systems. Currently, most of HBPs have been disposed by spreading on the pasture or dumping in the landfill. However, there is an increasing concern on environmental pollution by the disposal of HBPs. If a large amount of HBPs is applied to the soil, environmental pollutions in soil and water are increased (Tymczyna et al., 2000; Volterra and Conti, 2000). Therefore, the appropriate method of disposing HBPs without harming the environment should be developed.

The HBPs contain high amounts of protein (22 - 56%) and calcium (Ca) (1 - 29%) despite high variations in nutritional compositions. Accordingly, HBPs have gained an increasing attention as a potential ingredient in animal diets (Abiola and Onunkwor, 2004; Shahriar et al., 2008; Abiola et al., 2012). Especially, HBPs contain high amounts of total calcium (Ca) and phosphorus (P), such that inclusion of HBPs in animal diets can reduce the use of Ca and P supplements like limestones (i.e., calcium carbonate) and various calcium phosphate. However, few previous experiments have determined Ca and P utilization in the HBPs as an ingredient for animal diets. In addition, to our knowledge, no previous experiments have evaluated Ca and P utilization in individual HBPs including IFE, UHE, and LDC in animal diets. This lack of information regarding Ca and P utilization in HBPs has led animal nutritionists to hesitate to use HBPs in animal diets.

Therefore, the objective of the current experiment was to determine Ca and P utilization in 4 HBPs including IFE, UHE, LDC, and conventional MIX for broiler chickens as an animal model.

Materials and Methods

Preparation of hatchery by-products

The HBPs used in the current experiment were obtained from a local hatchery (Dongsan broiler hatchery, Cheonan, Korea). The collected HBP samples were separated to IFE, UHE, LDC, or HE. In addition, these HBP samples were mixed according to the proportion of production in the typical hatchery (55% of IFE, 10% of UHE and LDC, and 25% of eggshells) in South Korea to produce the MIX. Each of these 4 HBP samples (IFE, UHE, LDC, and MIX) was dried in a forced-air drying oven at 50°C for 24 h. All dried HBP samples were finely ground through a 1-mm screen grinder (CM 290 CemotecTM, FOSS, Hilleroed, Denmark) before the chemical analyses and metabolic trials.

The HBP samples were measured for gross energy (GE), dry matter (DM), crude ash, crude protein (CP), acid-hydrolyzed ether extract (AEE), Ca, P, and amino acids (AA) (Table 1). These samples were analyzed for DM (method 930.15; AOAC, 2007), crude ash (method 942.05; AOAC, 2007), CP (method 990.03; AOAC, 2007), AEE (method 996.01; AOAC, 2007), and GE using bomb calorimeter (Model 6400, Parr instruments Co., Moline, USA). Benzoic acid was used as the standard for calibration of GE analysis. The HBP samples were also measured for the concentrations of total Ca and P using an inductively coupled plasma spectrometer (ICP) (Optima 5300 DV, Perkin Elmer Inc., Shelton, USA). The concentrations of each AA in the HBP samples were also measured using the high performance liquid chromatography (HPLC; Oh et al., 2011) (Ultimate 3000, Thermo Dionex, Sunnyvale, USA).

Birds, diets, and experimental design

A total of 64 Ross 308 broiler chickens (50-d-old; initial BW = 2.36 ± 0.23 kg) were used. Birds were randomly allotted to 1 of 4 dietary treatments with 8 replicates per treatment. Two birds were placed together in one metabolic cage (35.2 cm × 45.0

cm × 55.3 cm, width × length × height). Room temperature was set at 20°C and the light was provided for 24 h during the entire experiment. Four treatments included 4 individual HBPs of IFE, UHE, LDC, or MIX.

A precision-fed chicken assay was conducted based on the methodology demonstrated in previous experiments (Kim et al., 2011; Lee et al., 2018). Briefly, broiler chickens were obtained at 1 d of age and were fed a commercial broiler diets until 50 d of age. All birds were provided with feed and water ad libitum before the precision-fed procedure began. At the start of the experiment (50 d of age), all birds were fasted for 12 h to empty the gastrointestinal tract (GIT). After 12 h-fasting, broiler chickens were fed 15 g of each HBP by crop intubation, and excreta were collected continuously for 48 h. Additional 16 birds were used to measure endogenous losses of Ca and P. Those birds were also fasted for 12 h and afterward excreta were collected for 48 h. All experimental procedures were reviewed and approved by the Institutional Animal Care and the Use Committee at Chung-Ang University.

Excreta collection and chemical analysis

Collected excreta samples were dried in a forced-air drying oven at 60°C for 48 h and finely ground for the further analysis.

Table 1. Energy and nutrient compositions of hatchery by-products (HBPs; as-fed basis).

Items	IFE	UHE	LDC	MIX
Dry matter (%)	98.3	97.8	96.6	98.0
GE (kcal/kg)	5,133	4,753	5,945	3,764
Crude protein (%)	34.2	39.4	60.0	31.2
AEE (%)	23.3	23.8	24.6	16.7
Crude ash (%)	27.6	27.6	6.6	40.6
Calcium (%)	8.88	10.47	1.25	17.16
Phosphorus (%)	0.49	0.50	0.77	0.31
Essential amino acid (%)				
Arginine	2.09	2.48	3.70	1.54
Histidine	0.84	0.99	1.37	0.64
Isoleucine	1.80	1.88	2.52	1.14
Leucine	3.01	3.25	4.55	1.94
Lysine	2.29	2.44	3.50	1.41
Methionine	1.10	1.10	1.37	0.70
Phenylalanine	2.00	2.03	2.86	1.26
Threonine	1.78	1.91	2.66	1.21
Tryptophan	0.39	0.51	0.63	0.37
Valine	2.27	2.41	3.26	1.51
Non-essential amino acid (%)				
Alanine	2.02	2.28	3.57	1.37
Aspartic acid	3.63	3.83	5.28	2.38
Cysteine	0.64	0.85	1.34	0.64
Glutamic acid	4.72	5.24	7.96	3.25
Glycine	1.28	1.88	4.13	1.27
Proline	1.44	1.85	3.41	1.24
Serine	2.59	2.67	3.48	1.70
Tyrosine	1.57	1.34	2.00	1.11

IFE, infertile eggs; UHE, unhatched eggs; LDC, low grade and dead chicks; MIX, mixture (55% IFE, 10% UHE, 10% LDC, and 25% eggshells); AEE, acid-hydrolyzed ether extract; GE, gross energy.

The excreta from additional 16 birds were also collected and pooled for measuring endogenous losses of Ca and P. Each of HBP samples and excreta samples were analyzed for Ca and P concentrations using ICP as demonstrated by Kurtoğlu et al. (2005).

Calculation

Apparent total tract retention (ATTR) and true total tract retention (TTTR) of Ca and P in 4 HBPs for broiler chickens were calculated as follows (Prola et al., 2013; Lee et al., 2018; Sun and Kim, 2018):

X ATTR = [(total X ingested – total X excreted) / total X ingested] × 100,

X TTTR = [(total X ingested – total X excreted + endogenous loss of X)

/ total X ingested] × 100

, where X represents Ca or P.

Statistical analysis

All data were analyzed by ANOVA as a completely randomized design using the GLM procedure of SAS (SAS Institute, Cary, USA). The replicate was used as the experimental unit for all analyses. Outlier data were checked using the UNIVARIATE procedure of SAS (Steel et al., 1997), but no outlier was identified. The means for ATTR and TTTR of Ca and P in various HBP were separated by LSD in multiple comparison procedure of SAS. Significance for statistical tests was set at $p < 0.05$.

Results and Discussion

The energy and nutrient compositions in 4 HBPs are presented in Table 1. For total Ca and P concentrations, total Ca concentrations of HBPs ranged from 1.25 to 17.16%. Those values agreed with the values from 1.04 to 25.62% reported by previous experiments (Abiola and Onunkwor, 2004; Mehdipour et al., 2009; Abiola et al., 2012). Likewise, total P concentrations of HBPs ranged from 0.31 to 0.77%. Those values were placed within the values from 0.01 to 0.84% reported by previous experiments (Rasool et al., 1999; Abiola and Onunkwor, 2004). These results indicate that HBPs used in this experiment are similar to those used in previous experiments. Interestingly, the MIX contained the greatest amounts of Ca, whereas LDC contained the least amounts of Ca. Other HBPs (IFE and UHE) contained intermediate amounts of Ca. In contrast, the LDC contained the greatest amounts of P, whereas the MIX contained the least amounts of P. The reason for these differences in total Ca and P concentrations is likely related to inclusion or presence of eggshells containing high amounts of Ca (36.65%) but low amounts of P (0.16%) (Gongruttananun, 2011). In the current experiment, the MIX was produced with additional eggshells but the LDC included few eggshells.

For the determination of Ca utilization and amounts of available Ca in the HBPs, the ATTR and TTTR of Ca in 4 HBPs were measured (Table 2). The ATTR of Ca in the IFE and MIX (57.0 and 61.4%, respectively) were greater ($p < 0.05$) than in UHE and LDC (44.6 and 35.2%, respectively). The values for TTTR of Ca were calculated by correcting ATTR of Ca with measured endogenous losses of Ca (0.118 g). The TTTR of Ca in the UHE (48.4%) was less ($p < 0.05$) than in the IFE, LDC, and MIX (61.4, 66.5, and 63.7%, respectively). The amount of available Ca in the HBPs was calculated with total Ca concentrations and TTTR of Ca. The amount of available Ca in the MIX (109.2 g/kg) was greater ($p < 0.05$) than those in the IFE and UHE (54.6 and 50.6 g/kg, respectively), which were greater ($p < 0.05$) than those in the LDC (8.3 g/kg).

Our values for the TTTR of Ca ranged from 48.4 to 66.5%. To our knowledge, no previous data are available on the TTTR of

Ca in individual HBP for broiler chickens. We found one experiment to determine the ATTR of Ca in eggshells for layer diets. Scheideler (1998) reported that the ATTR of Ca in eggshells ranged from 38.0 to 66.4% in laying hens. Moreover, the previous studies were performed to determine the TTTR of Ca in animal by-products, which is close to chemical compositions in HBPs. The TTTR of Ca in animal by-products ranged from 41.3 to 60.0% (Anwar et al., 2015, 2016). Thus, our values for TTTR of Ca in the HBPs were close to those values for eggshells and animal by-products from previous studies, indicating that the TTTR of Ca in HBPs were measured appropriately in the current experiment.

In the current experiment, the TTTR of Ca in IFE and LDC were greater than the TTTR of Ca in the UHE. The reason for the greater TTTR of Ca in the IFE than in the UHE is not clear; however, it may be related to different Ca formation between IFE and UHE. The Ca in IFE is present in eggs and eggshells, whereas Ca in the UHE is present in chicks' bones and eggshells. The Ca utilization in eggs may be greater than in chicks' bones, but no previous experiment measured the difference in Ca utilization between eggs and chicks' bones. However, the observation that Ca utilization was greater in the LDC than in the UHE may be more related to an over-estimation of Ca utilization in the LDC. This overestimation was attributed to the relatively large contribution of endogenous losses of Ca for total excretion of Ca in the LDC, which unexpectedly increased the TTTR of Ca. The TTTR of Ca in the MIX was similar to TTTR of Ca in the IFE, which may be a consequence of IFE being the main HBP in the MIX. The small mixture levels of UHE and LDC appears to have little effects on Ca utilization in the MIX. However, because of the highest concentrations of total Ca in the MIX, the amounts of available Ca in the MIX were the greatest among 4 HBP samples.

For the determination of P utilization and amounts of available P in the HBPs, the ATTR and TTTR of P in 4 HBPs were measured (Table 3). The ATTR of P in 4 HBPs were not different. The values for TTTR of P were calculated by correcting ATTR of P with measured endogenous losses of P (0.019 g). Likewise, TTTR of P in 4 HBPs were not different. However, the amount of available P in the LDC (6.5 g/kg) was greater ($p < 0.05$) than those in the IFE and UHE (4.3 and 4.2 g/kg, respectively), which were greater ($p < 0.05$) than those in the MIX (2.8 g/kg).

Our values for the TTTR of P ranged from 84.5 to 89.8%. To our knowledge, no published data are available on the TTTR of P in individual HBP for broiler chickens. However, some previous experiments measured the TTTR of P in the meat and bone meal, which contains similar chemical compositions of HBPs. The TTTR of P in the meat and bone meal ranged from 42.0 to 69.3% in broiler chickens (Mutucumarana et al., 2015; Mutucumarana and Ravindran, 2016). Sulabo and Stein (2013) also reported that the TTTR of P in the meat and bone meal ranged from 54.8 to 84.4% in pigs. The values for the TTTR of P in

Table 2. Calcium (Ca) utilization and amounts of available Ca in hatchery by-products (HBPs).

Items	Hatchery by-products (HBPs)				SEM	p-value
	IFE	UHE	LDC	MIX		
ATTR of Ca (%)	57.0a	44.6b	35.2b	61.4a	3.6	< 0.01
TTTR of Ca (%)	61.4a	48.4b	66.5a	63.7a	3.6	< 0.01
Total Ca in HBPs (g/kg)	88.8	104.7	12.5	171.6		
Available Ca[z] (g/kg)	54.6b	50.6b	8.3c	109.2a	3.4	< 0.01

IFE, infertile eggs; UHE, unhatched eggs; LDC, low grade and dead chicks; MIX, mixture (55% IFE, 10% UHE, 10% LDC, and 25% eggshells); ATTR, apparent total tract retention; TTTR, true total tract retention; SEM, standard error of mean.
[z]Amounts of available Ca (g/kg, as-fed basis) were calculated by multiplying TTTR of Ca (%) with total Ca concentrations (g/kg) in each HBP.
a - c: Means within a variable with no common superscript differ significantly ($p < 0.05$).

Table 3. Phosphorus (P) utilization and amounts of available P in hatchery by-products (HBPs).

Items	Hatchery by-products (HBPs)				SEM	p-value
	IFE	UHE	LDC	MIX		
ATTR of P (%)	74.3	72.5	76.3	69.7	1.8	0.10
TTTR of P (%)	87.2	85.2	84.5	89.8	1.8	0.20
Total P in HBPs (g/kg)	4.9	5.0	7.7	3.1		
Available P[z] (g/kg)	4.3b	4.2b	6.5a	2.8c	0.1	< 0.05

IFE, infertile eggs; UHE, unhatched eggs; LDC, low grade and dead chicks; MIX, mixture (55% IFE, 10% UHE, 10% LDC, and 25% eggshells); ATTR, apparent total tract retention; TTTR, true total tract retention; SEM, standard error of mean.
[z]Amounts of available P (g/kg, as-fed basis) were calculated by multiplying TTTR of P (%) with total P concentrations (g/kg) in each HBP.
a - c: Means within a variable with no common superscript differ significantly ($p < 0.05$).

the HBPs in the current experiment appeared to be greater than those for the TTTR of P in the meat and bone meal, which may indicate that P in eggs, eggshells, and chicks may be more utilizable than other types of animal products such as meat and bone meal. However, no experiments have been performed to prove this hypothesis.

The observation for no differences in the TTTR of P in the 4 HBPs in the current experiment indicates that P utilization in various HBPs (e.g., eggs, eggshells, and chicks) may be similar. The amount of available P was the greatest in the LDC, but the least in the MIX. This result is likely caused by differences in the concentrations of total P in 4 HBPs with similar TTTR of P among 4 HBPs.

One thing that should be noted in the current experiment was that we used relatively older broiler chickens (50-d-old). Many previous metabolism trials were conducted using younger broiler chickens aged from 21 d to 35 d. Thus, effects of broiler age on Ca and P utilization in HBPs may be possible; however, limited information regarding the effects of age on Ca and P utilization in broiler diets was available. Further researches are required to verify possible interactions between age and utilization of Ca and P in HBPs for broiler chickens.

Conclusion

Ca and P utilization in various HBPs is relatively higher as compared to other animal by-products. High concentrations of total Ca and P with high utilization rates signify that HBPs contain high amounts of available Ca and P. Therefore, the use of HBPs in broiler diets can reduce the inclusion of Ca and P supplements (i.e., calcium carbonate and various calcium phosphates).

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