Nutrient Digestibility and Mass Balance in Laying Hens Fed a Commercial or Acidifying Diet[1]

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ABSTRACT The objectives of the current study were to evaluate the effect of an acidifying diet (gypsum) combined with zeolite and slightly reduced crude protein (R) vs. a control diet (C) on nutrient retention in laying hens and compare 3 approaches to estimating nutrient excretion from hens: 1) mass balance calculation (feed nutrients – egg nutrient), 2) use of an indigestible marker with analyzed feed and excreta nutrient content, and 3) an environmental chamber that allowed for capturing all excreted and volatilized nutrients. Hens (n = 640) were allocated randomly to 8 environmental chambers for 3-wk periods. Excreta samples were collected at the end of each trial to estimate apparent retention of N, S, P, and Ca. No diet effects on apparent retention of N were observed (53.44%, $P > 0.05$). Apparent retention of S, P, and Ca decreased in hens fed R diet (18.7, −11.4, and 22.6%, respectively) compared with hens fed the C diet (40.7, 0.3, and 28.6%, respectively; $P < 0.05$). Total N excretion from hens fed the C and R diet was not different (1.16 g/hen/d); however, mass of chamber N remaining in excreta following the 3-wk period was less from hens fed the C diet (1.27 kg) than from hens fed the R diet (1.43 kg). Gaseous emissions of NH_3 over the 3-wk period from hens fed the C diet (0.74 kg per chamber) were greater than emissions from hens fed the R diet (0.45 kg). The 3-wk S excretion mass (estimated using the calculation, indigestible marker, and environmental chamber methods, respectively) was greater from hens fed the R diet (1.85, 1.54, and 1.27 kg, respectively) compared with hens fed the C diet (0.24, 0.20, and 0.14 kg, respectively). The 3-wk P excretion was similar between diets (0.68 kg). Results demonstrate that feeding the acidified diet resulted in decreased N emissions, but because of the acidulant fed, greatly increased S excretion and emissions.

Key words: laying hen, nitrogen, sulfur, mass balance, retention

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INTRODUCTION

Diet acidifiers have been used as a strategy to reduce N volatilization from excreta (McCrory and Hobbs, 2001; Kim et al., 2004). Gypsum is an acidogenic compound that has been used as a Ca source in laying hen diets without reducing laying hen performance (Keshavarz, 1991). A variety of additives adsorb NH_3, NH_4^+, or both; the most commonly used are zeolites, which exhibit a strong preference for binding nitrogenous cations such as NH_4^+, resulting in lower NH_3 concentration in excreta (Nakaue and Koelliker, 1981). Wu-Haan et al. (2007) reported that a reduced emissions diet containing gypsum, zeolites, and slightly reduced protein reduced emissions of NH_3, methane, and carbon dioxide and increased emissions of H_2S without affecting hen performance. However, it is not clear what effect this diet will have on nutrient retention and nutrient excretion.

The objectives of the current study were to evaluate the effect of an acidifying diet combined with zeolite and reduced crude protein on nutrient retention in laying hens and to compare 3 approaches for estimating nutrient excretion from hens: 1) mass balance calculation (feed nutrients – egg nutrient), 2) use of an indigestible marker with analyzed diet and excreta nutrient content, and 3) an environmental chamber that allowed for capturing all excreted and volatilized nutrients.

MATERIALS AND METHODS

Experimental Design

Animal-related experimental procedures were approved by the Iowa State University Committee for the Care and Use of Animals. The study consisted of 3 trials,

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each lasting 3 weeks, utilizing Hy-line W36 hens, starting at 21 (trial 1), 38 (trial 2), and 59 wk (trial 3) of age. All hens were obtained from high-rise laying hen houses (Rose Acres Farms, Stuart, IA), located 1 h away from the research location. During each trial, a total of 640 hens (initial BW = 1.36, 1.47, and 1.52 kg in trial 1, 2, and 3; respectively) were allocated randomly to 1 of 8 chambers (indirect calorimeters) and fed for a 3-wk period (ending age was 24, 41, and 62 wk for trial 1, 2, and 3, respectively). In each chamber, 80 birds were divided among four 2-cage units (10 birds per cage, 355 cm^2 of cage space per bird). Temperature in all chambers was maintained at 22 ± 2°C. Relative humidity ranged from 20 to 80%. Light (10 to 20 lx) was provided from 0600 to 1800 h for 21-wk-old birds and 0600 to 2200 h for the 38- and 59-wk-old birds. The light program was managed to mimic that of the commercial farm and meet the recommendations of the Hy-Line W36 Commercial Guide (Hy-line W36 Commercial Management Guide 2003-2005, Hy-Line International).

Diets

A reduced emission (**R**) or a control (**C**) diet was assigned randomly to each of the 8 chambers (4 chambers per diet) with the chamber constituting the experimental unit. The R diet was formulated at a reduced crude protein content and a 6.9% combination of $CaSO_4$ and zeolites, which replaced 35% of the limestone on a calcium content basis (Wu-Haan et al., 2007). On an analyzed basis, the R diet contained about 5 times more S than did the C diet as a result of additional gypsum. The R and C diets contained similar Ca, P, and energy. Both diets were formulated to meet or exceed NRC (1994) nutrient requirements and are described by Wu-Haan et al. (2007).

Apparent Retention of Nitrogen, Sulfur, Calcium, and Phosphorus

Animal performance measures are reported by Wu-Haan et al. (2007). Celite (World Minerals Inc., Santa Barbara, CA) was added to the diets at an inclusion level of 1% for 38 and 59 wk of age hens to serve as an indigestible marker. Feed samples were collected weekly and pooled to produce a single composite sample of each diet for each of the 3 trials. Twenty-four hour excreta collection samples were used for apparent retention determinations. The excreta samples were freeze-dried (–80°C), ground, and packaged in plastic bags, and stored (22°C) for analyses. Diet and excreta N, P, S, and Ca content were analyzed. Nitrogen was determined using the Kjeldahl method (AOAC, 1984). Diet and excreta total P were measured using the colorimetric molybdovanadate procedure (AOAC, 1984) and a Hach DR/4000 spectrophotometer (Hach Company, Loveland, CO). Sulfur content was determined using a Vario Max CNS machine (Elementar Corp., Mt. Laurel, NJ). Calcium was analyzed by the Central Analytical Laboratory at the University of Arkansas (Fayetteville) using an inductively coupled plasma technique. Acid-insoluble ash (**AIA**) was determined using the procedures described by Vogtmann et al. (1975).

Apparent retention was calculated as described by (Scott et al., 1976):

$$\text{Apparent retention of nutrient} = 100 - \{(\text{diet AIA}/\text{excreta AIA} \times \text{excreta nutrient content}/\text{dietary nutrient content}) \times 100\}.$$

Nutrient Mass Balance Determination

All excreta produced during each 3-wk period accumulated in the chamber, and the mass of excreta was measured at the end of each trial. A subsample of the excreta was collected for N, P, and S analyses using the methods described previously.

All N, S, and P inputs and outputs of the system were calculated. Nutrient inputs were constituted by the feed. Nutrient outputs included nutrients exported in eggs, excreta, and gaseous emissions to the atmosphere. Content of N, S, and P in a 60.8-g-of-weight egg (with shell)

Table 1. Apparent retention of excreta N, S, P, and Ca of hens fed a control (C) or reduced emission (R) diet at 41 and 62 wk of Hy-Line W36 age[1]

Item	Diet	N retention (%)	S retention (%)	P retention (%)	Ca retention (%)
Age wk)					
41	C	51.60	46.49	–3.85	37.66
	R	55.23	22.92	–13.39	19.32
62	C	58.62	34.94	4.45	26.58
	R	48.34	14.48	–9.41	18.66
Main effect					
Diet	C	55.11	40.71[a]	0.30[a]	28.59[a]
	R	51.78	18.70[b]	–11.41[b]	22.62[b]
Age (wk)	41	53.41	34.71[a]	–8.63	32.12[a]
	62	53.48	24.71[b]	–2.48	18.99[b]
SEM		7.178	5.117	8.459	4.827
Probability					
Diet		0.37	<0.01	0.02	0.04
Age		0.99	<0.01	0.17	<0.01
Diet × age		0.08	0.55	0.61	0.05

[a,b]Means within a column with different superscripts differ significantly ($P < 0.05$).

[1]Means represent 4 pens per diet at each age, 80 birds per pen.

was estimated at 1 g, 87.6 mg, and 126.1 mg, respectively (Cotterill et al., 1977; Stadelman and Pratt, 1989). Laying hens utilized in the current study were mature, and BW change was minimal; hence it was ignored. Mortality was 0.7% and ignored in the equation.

Three methods were compared for estimating nutrient excretions. The first method was a mass balance approach taking into account nutrient inputs and nutrients harvested as eggs. The calculated amount of excreta N (M_N), S (M_S), and P (M_P) excreted during the 3 trials (3 ages) was calculated as

$$M_{N,\,S,\,P} = (W_{feed} \times C_{feed}) - (W_{eggs} \times C_{eggs}). \quad [1]$$

The total weight of feed and eggs are represented as W_{feed} and W_{eggs}. Concentration of N, S, and P are represented as C_{feed} and C_{eggs} for feed and eggs, respectively.

The second method (marker) estimated excretions of N (E_N), S (E_S), and P (E_P) by hens during the 3-wk periods using an indigestible marker and analyzed feed and excreta nutrient content:

$$E_{N,\,S,\,P} = W_{excreta} \times C_{excreta}. \quad [2]$$

The $W_{excreta}$ represents the estimated total weight of excreta based on measured feed intake as well as feed and excreta concentrations of the indigestible marker. The $W_{excreta}$ was calculated as follows: $W_{excreta} = (AIA_{feed} \times W_{feed})/AIA_{excreta}$, where AIA_{feed} and $AIA_{excreta}$ represent the concentration of indigestible marker in feed and in the 24-h excreta sample, respectively, as measured in acid-insoluble ash. Concentration of N, S, and P of the 24-h excreta sample is represented by $C_{excreta}$.

The third method (chamber) involved calculating the mass of N (T_N), S (T_S), and P (T_P) excreted by hens during the 3-wk period by weighing the mass ($W_{excreta}$) and subsampling for nutrient analyses ($C_{excreta}$) and adding these to the mass of N and S emitted ($E_{N,S}$) to the atmosphere during the 3-wk period, based on measured emissions of NH_3, NO, NO_2, NO_2, and H_2S:

$$T_{N,\,S,\,P,} = W_{excreta} + E_{N,\,S}. \quad [3]$$

Detailed emissions data (NH_3, NO, NO_2, SO_2, and H_2S) are reported by Wu-Haan et al. (2007).

Statistical Analyses

Data were analyzed using a GLM procedure of SAS version 8.4 (SAS Institute, Cary, NC). The model included the fixed effects of diet (C and R diets), age, and the

Table 2. Composition (as fed-basis) of the reduced emission diet (R) and the control diet (C) fed to 21-, 38- and 59-wk-old laying hens[1]

	Laying hen age, wk					
	C diet			R diet		
Item	21 to 24	39 to 41	59 to 62	21 to 24	39 to 41	59 to 62
Ingredient, % of diet						
Corn	52.31	54.68	59.93	47.26	49.53	53.52
Soybean meal (48% CP)	30.07	27.09	23.16	29.67	26.65	23.74
Soy oil	5.16	4.90	3.73	7.19	6.97	6.00
Salt	0.41	0.41	0.41	0.41	0.41	0.41
DL-Methionine	0.20	0.16	0.09	0.22	0.18	0.11
Lysine	0.00	0.00	0.00	0.03	0.00	
Limestone	9.72	9.91	10.11	6.32	6.44	6.57
Dicalcium phosphate	1.78	1.50	1.22	1.78	1.50	1.22
Gypsum and zeolite mix[2]	0.00	0.00	0.00	6.80	6.94	7.08
Vitamin and mineral premix[3]	0.35	0.35	0.35	0.35	0.35	0.35
Celite	0.00	1.00	1.00	0.00	1.00	1.00
Nutrient composition (calculated)						
ME, kcal/kg	2,948	2,948	2,930	2,930	2,904	2,904
Protein, %	18.30	17.80	17.00	16.50	15.80	15.30
Ca, %	4.20	4.20	4.20	4.20	4.20	4.20
nPP, %	0.46	0.46	0.40	0.40	0.40	0.40
Met, %	0.48	0.49	0.43	0.44	0.35	0.35
Lys, %	1.02	0.99	0.93	0.93	0.83	0.83
TSAA, %	0.69	0.68	0.62	0.61	0.56	0.54
Analyzed composition						
Protein, %	18.0	17.0	16.2	17.0	15.5	15.6
S, %	0.25	0.20	0.20	0.99	1.20	1.10
Met, %	0.45	0.49	0.39	0.43	0.36	0.38
Lys, %	1.16	1.12	1.06	1.09	1.00	1.03

[1]Means represent 4 pens per diet at each age, 80 birds per pen.

[2]The gypsum and zeolite mixture is a patent-pending formulation thereby representing proprietary information.

[3]Vitamin mineral premix provided the following (per kg of diet): vitamin A, 12.3 KIU; vitamin D, 4.6 KIU; vitamin E, 15.4 KIU; vitamin K, 3.1 mg; riboflavin, 6.2 mg; pantothenic acid, 15.4 mg; niacin, 46.3 mg; menadione sodium bisulfate complex, 1.0 mg; choline chloride, 463.1 mg; folic acid, 0.3 mg; vitamin B_{12}, 23.1 μg; zinc as zinc oxide, 71.4 mg, iron as ferrous sulfate, 50.4 mg; manganese as manganese oxide, 89.6 mg; copper as copper sulfate, 7 mg; iodine from ethylene diamine dihydroiodide, 0.7 mg; selenium as sodium selenite, 0.42 mg.

interaction between age and diet. Significant differences among the means were declared at $P < 0.05$.

RESULTS AND DISCUSSION

Performance measures are reported by Wu-Haan et al. (2007). Across ages, the average daily egg weight (56.3 g), average daily egg production (81.0%), and average daily feed intake (92.4 g/hen/d) of hens fed the R diet were not different from hens fed the C diet.

Apparent Retention of Nitrogen, Sulfur, Calcium, and Phosphorus

Apparent retention, based on marker method of N is presented in Table 1. Across ages, apparent retention of N in hens fed the R diet (51.78%) was not different from hens fed the C diet (55.11%). Apparent retention of N in hens at 41 and 62 wk was 53.41 and 53.48%, respectively, with no age or diet × age interaction effects observed. These results are similar to work by Keshavarz and Austic (2004) who reported that apparent retention of N was 48.8% when hens were fed a diet containing 16.5% protein. The difference in retention values between Keshavarz and Austic (2004) and the current study are possibly due to ingredients utilized in the diets and the method used to determine retention (ileal vs. excreta sampling).

Apparent retention of S is presented in Table 1. Sulfur retention was affected by diet and hen age. Across ages, apparent retention of S decreased in hens fed the R diet (18.70%) compared with hens fed the C diet (40.71%). Hens at 41 wk (34.71%) had greater apparent retention of S than hens at 62 wk of age (24.71%). Gypsum, a sulfur-containing compound, was added to the diet as an acidifying agent, which led to greater S excretion and likely caused reduced digestive efficiency.

Across ages, apparent retention of P was lower in hens fed the R diet (–11.41%) than hens fed the C diet (0.30%; Table 2). No age effect was observed. The negative retention is much lower than previously reported values (15 to 40%; Carlos and Edwards, 1998; Um and Paik, 1999) and is likely a reflection of the relatively high concentration of P in the diet (average 0.63%) combined with the unaccounted part of the P balance equation related to bone mineralization/demineralization.

Apparent retention of Ca is also presented in Table 1. Across ages, apparent retention of Ca was reduced in hens fed the R diet (22.6%) compared with hens fed the C diet (28.6%). These results are similar to that reported by Jalal and Scheideler (2001) who demonstrated a Ca

Table 3. Chamber mass balance of nitrogen (N) during a 3-wk period of hens (80 hens per chamber) fed an acidifying diet supplemented with gypsum, zeolites, and slightly reduced crude protein diet (R) at 21, 38, and 59 wk of age compared to commercial diet (C)[1]

		Nitrogen (kg)						
Item	Diet	Feed	Egg	Calculated excretion[2]	Excreta[3]	Emission[4]	Estimated excretion[5]	Measured excretion[6]
Age (wk)								
21 to 24	C	4.19	1.22	2.97	1.77	0.41	—	2.18
	R	3.94	1.23	2.71	1.82	0.30	—	2.11
38 to 41	C	4.16	1.40	2.76	1.01	0.88	2.01	1.89
	R	4.00	1.36	2.64	1.26	0.49	1.79	1.74
59 to 61	C	4.24	1.19	3.05	1.04	0.93	1.75	1.97
	R	3.99	1.23	2.76	1.23	0.58	2.06	1.82
Main effect mean								
Diet								
C		4.20	1.27	2.93	1.27	0.74	1.88	2.01
R		3.98	1.27	2.70	1.43	0.45	1.92	1.89
Age (wk)								
21 to 24		4.07	1.22	2.84	1.79	0.35	—	2.15
38 to 41		4.08	1.38	2.70	1.13	0.68	1.90	1.82
59 to 61		4.12	1.21	2.90	1.14	0.75	1.91	1.89
SEM		0.061	0.035	0.065	0.185	0.050	0.292	0.191
Probability								
Diet		<0.01	0.81	<0.01	0.04	<0.01	0.76	0.15
Age		0.28	<0.01	<0.01	<0.01	<0.01	0.97	<0.01
Diet × age		0.24	0.14	0.05	0.54	<0.01	0.09	0.88

[1]Means represent 4 pens per diet at each age, 80 birds per pen.

[2]Calculated excretion = ($W_{feed} \times C_{feed}$) – ($W_{eggs} \times C_{eggs}$). The total weight of feed and eggs are represented as W_{feed} and W_{eggs}; concentration of N is represented as C_{feed}, and C_{eggs} for feed and eggs, respectively. The N content of egg is 1.7% (Cotterill et al., 1977; Stadelman and Pratt, 1989).

[3]Excreta N content after 3-wk period ($C_{excreta}$).

[4]Emissions of NH_3, NO, and NO_2. Emissions of N_2 and N_2O were not measured.

[5]Estimated N excretion during a 3-wk period = $W_{excreta} \times C_{excreta}$; the $W_{excreta}$ represents the estimated total weight of excreta and calculated by the following equations: $W_{excreta} = (AIA_{feed} \times W_{feed})/AIA_{excreta}$. The AIA_{feed} and $AIA_{excreta}$ represent the percentage of acid insoluble ash of feed and 24-hr excreta sample, respectively. $C_{excreta}$ represents the concentration of N of 24-hr excreta sample. Celite (1%) was only added to the diets of 38- and 59-wk-of-age hens; thus, data for 21-wk-of-age hens were not available.

[6]The measured excretion = $W_{excreta} \times C_{excreta} + N_{emitted}$, where W is a measured variable.

Table 4. Chamber mass balance of S during a 3-wk period of hens (80 hens per chamber) fed an acidifying diet supplemented with gypsum, zeolites, and slightly reduced crude protein diet (R) at 21-, 38-, and 59-wk of age compared with the commercial diet (C)[1]

		S (kg)						
Item	Diet	Feed	Egg	Calculated excretion[2]	Excreta[3]	Emission[4]	Estimated excretion[5]	Measured excretion[6]
Age								
21 to 24	C	0.38	0.10	0.28	0.15	0.70	—	0.16
	R	2.06	0.10	1.96	1.39	2.41	—	1.39
38 to 41	C	0.36	0.12	0.25	0.12	3.25	0.19	0.12
	R	1.92	0.11	1.81	1.29	12.23	1.48	1.30
59 to 61	C	0.31	0.10	0.21	0.14	1.72	0.20	0.14
	R	1.88	0.10	1.77	1.11	5.47	1.60	1.12
Main effect mean								
Diet								
C		0.35	0.10	0.24	0.14	1.89	0.20	0.14
R		1.95	0.10	1.85	1.26	6.70	1.54	1.27
Age								
21 to 24		1.22	0.10	1.12	0.77	1.55	—	0.77
38 to 41		1.14	0.11	1.03	0.70	7.74	0.84	0.71
59 to 61		1.09	0.10	0.99	0.63	3.59	0.90	0.63
SEM		0.015	0.003	0.014	0.095	0.852	0.064	0.095
Probability								
Diet		<0.01	0.81	<0.01	<0.01	<0.01	<0.01	<0.01
Age		<0.01	<0.01	<0.01	0.02	<0.01	0.06	0.03
Diet × age		<0.01	0.14	<0.01	0.04	<0.01	0.09	0.04

[1]Means represent 4 pens per diet at each age, 80 birds per pen.

[2]Calculated excretion = ($W_{feed} \times C_{feed}$) − ($W_{eggs} \times C_{eggs}$). The total weight of feed and eggs are represented as W_{feed} and W_{eggs}; concentration of S is represented as C_{feed}, and C_{eggs} for feed and eggs, respectively. The S content of egg is 0.14% (Cotterill et al., 1977; Stadelman and Pratt, 1989).

[3]Excreta S content after 3-wk period ($C_{excreta}$).

[4]Emissions of H_2S.

[5]Estimated S excretion during a 3-wk period = $W_{excreta} \times C_{excreta}$. The $W_{excreta}$ represents the estimated total weight of excreta and calculated by the following equations: $W_{excreta} = (AIA_{feed} \times W_{feed}.)/AIA_{excreta}$. The AIA_{feed} and $AIA_{excreta}$ represent the percentage of acid insoluble ash of feed and 24-hr excreta sample, respectively. $C_{excreta}$ represents the concentration of S in a 24-hr excreta sample. Celite (1%) was only added to the diets of 38- and 59-wk-of-age hens; thus, data for 21-wk-of-age hens were not available.

[6]The measured excretion = $W_{excreta} \times C_{excreta} + S_{emitted}$, where W is a measured variable.

retention of 29.3%. The acidifying diet likely shifted the acid-base balance of hen's blood system resulting in greater Ca excretion (Keshavarz, 1991) and reduced digestive efficiency in hens fed the R diet.

Nutrient Mass Balance

On an analyzed basis, across diet and age, the concentrations of N, S, and P of the 24-h excreta samples were 4.61, 1.74, and 1.98% (DM basis), respectively. Across diet and age the concentrations of excreta N, S, and P that were present in the excreta accumulated during the 3-wk period trial were 3.96, 2.02, and 1.99% (DM basis). The increased concentration of S but not of N or P in the 24-h sample compared with the cumulative sample is difficult to explain given that excreta did not dry considerably during storage and that N and S volatilized. Analytical error combined with sampling error is the most likely explanation. Sampling error would include feathers in the accumulated sample but not in the 24-h sample.

Chamber N mass balance is presented in Table 3. Nitrogen intake was greater in hens fed the C diet (4.20 kg) than in hens fed the R diet (3.98 kg) because of the higher dietary protein concentration of the C diet given that feed intake was similar (92.1 and 92.7 g/hen/d for hens fed the C and R diets, respectively) in both diets (Wu-Haan et al., 2007). The N content of eggs with shells has been previously estimated as 1.7% (Cotterill et al., 1977; Stadelman and Pratt, 1989), and these values were used to calculate the mass of N that left the system in the form of egg during the course of the trial. Total N content of eggs produced by hens over the course of the 21-d housing period was assumed to be similar for hens fed the C and R diets (1.27 kg), based on no differences in egg production (Wu-Haan et al., 2007). Age did affect egg production (Wu-Haan et al., 2007); as a result of this the N exported in eggs was greater for hens at 41 wk (1.38 kg) of age than for hens at 24 (1.22 kg) and 62 wk (1.21 kg) of age. The N content of excreta accumulated during the 3-wk trial period for hens fed the C diet (1.27 kg) was less than for hens fed the R diet (1.43 kg). However, emissions of NH_3 from hens fed the C diet (0.74 kg) were greater than emissions from hens fed the R diet (0.45 kg), likely the result of the R diet's ability to trap N via acidification, resulting in less NH_3 volatilization.

Nitrogen excretion data were estimated using 3 methods: mass balance calculation (feed - egg), an indigestible marker combined with concentration measures, and use of the environmental chamber to capture all emissions (equations [1], [2], and [3], respectively). Using equation

Table 5. Chamber mass balance of P during a 3-wk period of hens (80 hens per chamber) fed an acidifying diet supplemented with gypsum, zeolites, and slightly reduced crude protein diet at 21, 38, and 59 wk of age compared with the commercial diet (C)[1]

		P (kg)					
Item	Diet	Feed	Egg	Calculated excretion[2]	Excreta[3]	Estimated excretion[4]	Measured excretion[5]
Age (wk)							
21 to 24	C	0.87	0.11	0.76	0.78	—	0.78
	R	0.83	0.11	0.72	0.86	—	0.86
38 to 41	C	0.86	0.12	0.73	0.66	0.89	0.66
	R	0.87	0.12	0.75	0.63	0.99	0.63
59 to 61	C	0.73	0.11	0.63	0.54	0.70	0.54
	R	0.77	0.11	0.66	0.58	0.84	0.58
Main effect mean							
Diet							
C		0.82	0.11	0.71	0.66	0.79	0.66
R		0.82	0.11	0.71	0.69	0.92	0.69
Age (wk)							
21 to 24		0.85	0.11	0.74	0.82	—	0.82
38 to 41		0.86	0.12	0.74	0.65	0.94	0.65
59 to 61		0.75	0.11	0.65	0.56	0.77	0.56
SEM		0.011	0.004	0.012	0.085	0.076	0.085
Statistic probability							
Diet		0.33	0.81	0.39	0.36	<0.01	0.36
Age		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diet × age		<0.01	0.14	<0.01	0.43	0.53	0.43

[1]Means represent 4 pens per diet at each age, 80 birds per pen.

[2]Calculated excretion = $(W_{feed} \times C_{feed}) - (W_{eggs} \times C_{eggs})$. The total weight of feed and eggs are represented as W_{feed} and W_{eggs}; concentration of P is represented as C_{feed}, and C_{eggs} for feed and eggs, respectively. The P content of egg is 0.21% (Cotterill et al., 1977; Stadelman and Pratt, 1989).

[3]Excreta P content after 3-wk period ($C_{excreta}$).

[4]Estimated P excretion during a 3-wk period = $W_{excreta} \times C_{excreta}$; the $W_{excreta}$ represents the estimated total weight of excreta as calculated by the following equations: $W_{excreta} = (AIA_{feed} \times W_{feed})/AIA_{excreta}$. The AIA_{feed} and $AIA_{excreta}$ represent the percentage of acid insoluble ash of feed and 24-hr excreta sample, respectively. $C_{excreta}$ represents the concentration of P in a 24-hr excreta sample. Celite (1%) was only added to the diets of 38- and 59-wk-of-age hens; thus, data for 21-wk-of-age hens were not available.

[5] The measured excretion = $W_{excreta} \times C_{excreta}$, where W is a measured variable.

[1], N excretion from hens fed the C and R diets were 2.93 and 2.70 kg, respectively, over the 3-wk trial period. Estimated N excretion using equation [2] was 1.88 and 1.92 kg from hens fed the C and R diets, respectively. Measured N excretion after the 3-wk accumulation period (equation [3]) resulted in an estimated 2.01 kg of N excretion from hens fed the C diet and 1.89 kg from hens fed the R diet. Using equation [2] (marker method) resulted in a N excretion value that was only 69.3% of the value that resulted from using the mass balance approach (equation [1]) but was similar to the value obtained using equation [3] (environmental chamber). Differences in values that resulted from application of the 3 different equations could be due to the N lost during sampling and storage (such as dust, feather) that was not considered in the mass balance calculation. The emissions of N_2 and N_2O were not measured because of equipment limitations; however, the contribution of these 2 gases is likely small. Dinitrogen gas emissions have been reported as significant contributors to N losses from anaerobic lagoons as a result of chemical, or nonbiological, denitrification (Harper et al., 2004). Harper et al. (2004) theorized that NH_4^+ oxidizes to N_2 at very low O_2 pressures. In their work, Harper et al. (2004) estimate that as much as 43% of N entering the farm was ultimately lost as N_2. Nitrous oxide would form also as the result of anaerobic conditions and is highly unstable. Harper et al. (2004) measured N_2O emissions from swine lagoons and estimated N_2O emissions as representing 0.1% of N inputs into the farm system.

Chamber mass balance of S data is presented in Table 4. Sulfur intake was greater for hens fed the R diet (1.95 kg) than hens fed the C diet (0.35 kg) due to the addition of sulfur ($CaSO_4$) to the R diet. The sulfur content of eggs has been previously estimated as 0.14% (Cotterill et al., 1977; Stadelman and Pratt, 1989), and this value was used in estimating the mass of S removed from the system in eggs over the course of the trial. The S content of excreta after 3 wk of storage was lower for hens fed the C diet (0.14 kg) than hens fed the R diet (1.26 kg). Emissions of H_2S from hens fed the C diet (1.89 g) were also less than emissions from hens fed the R diet (6.70 g). Using equations [1], [2], and [3], S excretion over the 3-wk period was greater from hens fed the R diet (1.85, 1.54, and 1.27 kg, respectively) compared with hens fed the C diet (0.24, 0.20, and 0.14 kg, respectively). Using equation [2] (marker method) resulted in an S excretion value that was only 83% of the value that resulted from using the mass balance approach (equation 1) and 123% of the value obtained using equation [3] (environmental chamber). The mass balance approach may reflect outdated values of S for egg composition, thereby overestimating S retention, whereas the marker method did not account for the S content of feathers because feathers were excluded from the 24-h sample used in the marker method (equation [2]) but were present on the accumulated sample used with equation [3].

Chamber mass balance of P data are presented in Table 5. Phosphorus contents of feed consumed, eggs, and excreta were similar between hens fed the C diet (0.82, 0.11, and 0.66 kg, respectively) and hens fed the R diet (0.86, 0.11, and 0.69 kg, respectively). The average P excretions from hens fed both diets was equal to 0.40 g/hen/d. Age significantly affected P excretion. These results could be due to dietary P supplement differences at 21, 38, 59 wk of age relative to bird requirements. The P content of excreta at the end of the 3-wk excreta accumulation period was similar from hens fed the C (0.66 kg) and R diet (0.69 kg). Using equation [1], the calculated P excretions were the same (0.67 kg) from hens fed the R diet compared with hens fed the C diet. When the indigestible marker method was used (equation [2]), the estimated P excretions were greater from hens fed the R diet (0.92 kg) than those fed the C diet (0.72 kg). The measured P excretion using the chamber method was similar from hens fed the C (0.66 kg) and the R (0.69 kg). Using equation [2] (marker method) resulted in a P excretion value that was 122% of the value that resulted from using the mass balance approach (equation [1]) or the environmental chamber approach (equation [3]).

Because P does not volatilize it was considered as a marker to evaluate the recovery from the environmental chambers. The findings demonstrate that the chamber method effectively accounted for nutrient excretions estimated using the mass balance method. Differences observed among the 3 methods of estimating nutrient excretion are likely attributed to losses of nutrients in feathers and feed dust, changes in BW and mortality, and sampling and equipment errors. For N and S, the estimated excretion method, based on 24-h collection of excreta when a marker was fed, was the closest to actual measured excretions using the chamber method.

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