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Abstract

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Keywords

drying method, feces, poultry excreta, swine, urine

Disciplines

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Effects of drying methods on nitrogen and energy concentrations in pig feces and urine, and poultry excreta¹

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ABSTRACT: Accurate estimations of nutrient digestion and retention are critical in nutrient balance and feed evaluation studies because errors that occur are often additive. However, there is no standard universal method for drying feces, urine, or excreta before laboratory analysis. The objective of this study was to evaluate the impact of 4 different drying methods on nutrient concentrations in feces, urine, and excreta. Twelve individually penned growing pigs were fed 1 of 3 diets and 16 pens of 10 growing broilers were fed 1 of 4 diets that differed in NDF and CP. Feces, urine, and excreta that varied in nutrient composition were collected after 7 d of diet adaptation. Samples were dried using 1 of 4 methods: undried (UD), freeze-dried (FD), oven-dried at 55°C for 48 h (OD55), or oven-dried at 100°C for 48 h (OD100), after which DM, GE, N, C, and S were determined. In swine feces, drying resulted in a loss of GE

($P < 0.10$) and S ($P < 0.01$) by 5 and 58%, respectively, compared with UD feces. There was no difference ($P \geq 0.36$) among drying method on DM, GE, N, C, or S concentrations. There were no differences ($P \geq 0.12$) in urinary GE due to drying or between drying methods; however, urinary DM was greatest by FD compared with OD ($P < 0.05$) and greater for OD55 compared with OD100 ($P < 0.01$). In poultry excreta, GE ($P < 0.05$), N ($P < 0.10$), and S ($P < 0.01$) were reduced by drying by an average of 6, 10, and 66%, respectively. There were no differences ($P \geq 0.50$) among drying methods except FD excreta had a greater S concentration than OD ($P < 0.10$). Regardless of drying method, some GE and N loss appears to be inevitable, but there is no apparent advantage between FD and OD. The apparent greater S losses warrant further investigation.

Key words: drying method, feces, poultry excreta, swine, urine

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INTRODUCTION

Accurate nutrient estimation in urine, feces, and excreta is critical for nutrient digestibility studies (Just et al., 1982; van Kempen et al., 2003; Kerr et al., 2010). Currently, no standard method of drying feces, urine, or excreta for determination of energy or N concentration has been accepted in the scientific community (Bach Knudsen and Hansen, 1991; Pedersen et al., 2007; Kerr et al., 2009). In poultry, Manoukas et al. (1964)

demonstrated that oven drying (OD) at 65°C resulted in energy and N losses of 11.4 and 5.2%, respectively, compared with fresh, undried (UD) excreta. Shannon and Brown (1969) reported energy losses were least (1.3%) with freeze drying (FD), whereas losses of energy decreased with increasing OD temperature (5.5% at 60°C, 3.3% at 100°C, and 2.8% at 120°C) compared with UD excreta. For N, FD or OD at 60°C resulted in a loss of approximately 4.7%, whereas increasing the temperature to 100 or 120°C increased losses to 7.8 and 10.5%, respectively. Ribeiro et al. (2001) reported that increasing oven temperature from 55 to 100°C increased N losses from 8.3 to 13.2%, respectively, relative to UD excreta. Wallis and Balnave (1983) reported greater energy and N losses when excreta were FD compared with OD at 60 or 80°C. Dale et al. (1985) found no significant differences in the true ME when poultry excreta samples were FD or OD at 60°C, whereas Jorgensen et al. (1984) reported that method of fecal preparation for N analysis (UD, FD, or OD at 70°C) did not affect protein digestibility measurements. Because there

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Table 1. Composition of swine diets, as-fed basis

Item	Dehulled, degermed corn	Corn-soybean meal	Dried distillers grains with solubles
Ingredient, %			
Corn	—	78.40	56.70
Soybean meal	18.00	18.00	15.00
Dehulled, degermed corn	78.19	—	—
Dried distillers grains with solubles	—	—	25.00
Soybean oil	0.50	0.50	0.50
L-Lysine-HCl	0.11	—	—
Dicalcium phosphate	0.85	0.70	0.15
Limestone	0.70	0.75	1.00
Sodium chloride	0.50	0.50	0.50
Vitamin premix ¹	0.05	0.05	0.05
Trace mineral premix ²	0.05	0.05	0.05
Titanium dioxide	0.50	0.50	0.50
Clay ³	0.50	0.50	0.50
Antibiotic ⁴	0.05	0.05	0.05
Calculated composition			
ME, kcal/kg	3,293	3,332	3,193
CP, %	14.27	15.06	18.76
NDF, %	4.50	9.10	15.40
Crude fat, %	1.20	4.10	5.30
Phosphorus, %	0.34	0.47	0.48

¹Supplied per kilogram of diet: vitamin A, 6,600 IU; vitamin D₃, 880 IU; vitamin E, 44 IU; vitamin K (menadione sodium bisulfate complex), 6.4 mg; thiamin, 4.0 mg; riboflavin, 8.8 mg; pyridoxine, 4.4 mg; vitamin B₁₂, 33 µg; folic acid, 1.3 mg; niacin, 44 mg; pantothenic acid, 22 mg; D-biotin, 0.22 mg.

²Supplied per kilogram of diet: Zn, 131 mg as ZnO; Fe, 131 mg as FeSO₄·H₂O; Mn, 45 mg as MnO; Cu, 13 mg as CuSO₄·5H₂O; I, 1.5 mg as CaI₂O₆; Co, 0.23 mg as CoCO₃; Se, 0.28 mg as NaSeO₃.

³AB-20, Prince Agriproducts, Quincy, IL.

⁴Tylan-40 supplied at 44 mg/kg of diet, Elanco, Greenfield, IN.

is no consensus regarding the impact of drying on energy and N losses, additional information is needed. The objective of this study was to determine how each of 3 drying methods [OD at 55° or 100°C; **OD55** and **OD100**, respectively] and FD] compared with UD on DM, GE, N, C, and S concentrations of swine feces and urine, and poultry excreta.

MATERIALS AND METHODS

The Institutional Animal Care and Use committee at Iowa State University and Auburn University approved all experimental protocols for the swine and poultry research, respectively.

Pig Sample Management

Experimental diets (Table 1) were mixed at the University of Kentucky (Lexington), and were formulated to contain varying amounts of CP (15.06 to 18.76%) and NDF (4.5 to 15.4%). Three blocks of 4 gilts (initial BW of 47.1 kg, Landrace × Large White × Duroc cross) were placed in individual pens (0.57 × 2.21 m) and randomly assigned to dietary treatments. Pigs were offered water and feed for ad libitum intake and were weighed at the beginning and end of the experiment. Pigs were allowed 7 d for adaptation to the diet before urine and fecal collections. After the adaptation period, urine and fecal grab samples from each pig were obtained twice daily, 0700 and 1800 h, for the sub-

sequent 10 d to ensure adequate sample volumes for laboratory analysis. Fresh urine was collected daily in plastic containers without the addition of acid, whereas feces were collected in sealable plastic bags and immediately stored at 10°C. After the 10 d of collection, samples were pooled within pig and stored at -20°C until further analysis.

Feces were thawed, homogenized, and subsequently subdivided to accomplish the different drying methods after thorough mixing. Oven-dried samples were placed in a forced-air oven either at 55 or 100°C (±2°C) for 48 h (**OD55** or **OD100**, respectively). Lyophilization was achieved in a freeze drier at -80°C for 24 h and then placed in vacuum tubes, where the samples were cooled to -110°C under a vacuum pressure of ≤100 µm for 48 h. Urine samples were thawed in a refrigerator, mixed, and separated into 2 categories, acidified and unacidified. After thawing, acidification took place immediately by adding 1.5 mL of 6 M HCl to decrease the initial pH to 2 to minimize the potential of bacterial growth, whereas the unacidified samples were not treated. Urine was enclosed in plastic screw-top containers and refrigerated at all times before DM, GE, N, S, and C analyses. Drying method was a factor in urine GE determination, but due to equipment used in the current experiment (thermocombustion), N, S, and C were analyzed only as UD. For GE analysis, cellulose pellets were used to absorb and subsequently dry the urine for the OD methods, whereas cotton balls and small plastic bags (Jeb Plastics Inc., Wilmington, DE)

were used to absorb urine in the crucible for the FD method. In both instances, samples were allowed to dry for 48 h. The oven varied by $\pm 1^\circ\text{C}$ during times of drying, and the freeze drier varied $\pm 2^\circ\text{C}$.

Poultry Sample Management

Experimental diets used were formulated and mixed at Auburn University, AL as described in Table 2 that ranged in CP (17.85 to 26.95%) and NDF (7.62 to 15.78%). Broilers (160 Ross \times Ross 708, 10 per pen; 5 males and 5 females) were fed a common diet for ad libitum intake from d 1 to 15 and then randomly assigned to 1 of 4 dietary treatments from d 15 to 22 (4 replicate pens per diet). Broilers were offered feed and water ad libitum. Excreta samples were collected on pans under the pens on d 23 and 24, subsequently stored at -20°C for later analysis, after which they were thawed and homogenized before drying method and chemical analyses.

Chemical Analyses

Percentage DM was determined by measuring the weight of samples before and after the completion of each respective drying method. Dried fecal and excreta samples were ground through a 1-mm screen in preparation for chemical composition determination. For UD urine GE concentrations, 1 mL of urine was added to 0.5 g of dried cellulose and then analyzed for GE. For

urine dried at 55 and 100°C , 3 mL of urine was added to 0.5 g of dried cellulose and dried for 24 h under the respective drying temperature before determining GE. Freeze-dried urine samples were analyzed by the addition of 2 mL of urine to a 0.15-g dried cotton ball and frozen for 24 h at -80°C , after which the samples were placed under the freeze-drying vacuum conditions for 24 h. Gross energy of feces, urine, and excreta was determined by the use of an isoperibol bomb calorimeter (model 1281, Parr Instrument Co., Moline, IL), with benzoic acid in the samples for a standard. In all cases, the GE of the cellulose or plastic bag plus cotton ball was subtracted from the total energy (urine plus carrier) to determine urinary GE. To maintain a laboratory CV below 5% for these analyses, duplicate analyses were performed on all fecal and excreta samples from each pig or pen of broilers, whereas urine samples were analyzed in triplicate on cellulose or cotton balls. Nitrogen and C were analyzed by thermocombustion (Vari-oMax, Elementar Analysensysteme, GmbH, Hanau, Germany), which uses catalytic tube combustion to volatilize the sample. Percentage DM, C, N, and S, and kilocalories of GE were calculated for feces, urine, and excreta on an UD basis (Tables 3, 4, and 5).

Statistic Analysis

Data were analyzed using PROC MIXED (SAS Inst. Inc., Cary, NC) with the individual pig or the pen of broilers defining the experimental unit. The statisti-

Table 2. Composition of broiler diets, as-fed basis

Item	Dextrose	Corn gluten meal	Dried distillers grains with solubles	Corn germ meal
Ingredient, %				
Corn	52.99	52.99	52.99	52.99
Soybean meal	27.95	27.95	27.95	27.95
Dextrose	15.00	—	—	—
Corn germ meal	—	—	—	15.00
Corn gluten meal	—	15.00	—	—
Dried distillers grains with solubles	—	—	15.00	—
Dicalcium phosphate	1.46	1.46	1.46	1.46
Limestone	0.96	0.96	0.96	0.96
DL-methionine	0.24	0.24	0.24	0.24
Sodium chloride	0.44	0.44	0.44	0.44
Vitamin premix ¹	0.21	0.21	0.21	0.21
Mineral premix ²	0.21	0.21	0.21	0.21
Coccidostat ³	0.04	0.04	0.04	0.04
Titanium dioxide	0.50	0.50	0.50	0.50
Calculated composition				
ME, kcal/kg	3,020	2,910	2,833	2,740
CP, %	17.85	26.95	21.87	21.02
NDF, %	7.62	9.30	13.05	15.78
Crude fat, %	2.92	3.11	4.40	3.24
Phosphorus, %	0.21	0.30	0.33	0.30

¹Supplied per kilogram of diet: vitamin A, 8,000 IU; vitamin D, 2,000 IU; vitamin E, 8 IU; vitamin K (menadione sodium bisulfate complex), 2 mg; vitamin B₁₂, 0.02 mg; folic acid, 0.5 mg; D-pantothenic acid, 15 mg; riboflavin, 5.4 mg; niacin, 45 mg; thiamine, 1 mg; biotin, 0.05 mg; pyridoxine, 2.2 mg; choline, 500 mg.

²Supplied per kilogram of diet: Mn, 65 mg as MnO; Zn, 55 mg as ZnO; Fe, 55 mg as FeSO₄; Cu, 6 mg as CuSO₄·5H₂O, I, 1 mg CaI₂O₆; Se, 0.3 mg as NaSeO₃.

³Bio-Cox 60 provided 53 mg of salinomycin sodium/kg of diet, Alpharma, Fort Lee, NJ.

Table 3. The effect of drying method on the composition of pig feces, as-is basis¹

Analyses	Drying method ²				Model ³		Contrast ⁴		
	UD	FD	OD55	OD100	SEM	<i>P</i> -value	UD vs. dry	FD vs. OD	OD55 vs. OD100
DM, %	—	31.04	31.17	32.46	3.01	0.93	—	0.86	0.76
GE, cal/g	1,374	1,297	1,315	1,293	33	0.28	0.06	0.86	0.64
N, %	1.31	1.24	1.23	1.29	0.10	0.93	0.63	0.86	0.66
C, %	13.10	12.25	12.42	13.34	0.96	0.83	0.70	0.59	0.51
S, %	4.68	1.45	2.17	2.22	0.65	0.01	0.01	0.36	0.96

¹Fresh fecal matter collected from growing pigs with 12 observations per drying method.

²Drying methods consisted of undried (UD), freeze drying (FD), and oven drying at 55°C (OD55) or 100°C (OD100).

³Model statistics.

⁴Preplanned contrast statements.

cal model for feces and urine drying included method, diet, and pig as fixed effects; with group added as an additional fixed effect in analyzing the poultry data. Method and diet within pig were random effects for the swine data; and group, chicken within group, and method within chicken were random effects for the poultry data. To test the effect of urinary acidification on DM, GE, N, C, or S concentrations, acidification was added as a fixed effect, but because there was no effect, it was not included in the final model. Means are reported as least squares means. Data are discussed relative to a protected *F*-test and an unprotected *F*-test using preplanned contrasts between the UD and dried samples, between FD and OD, and between OD at 55 or at 100°C. The use of both statistical analyses provides an unbiased discussion of the data (Barnette and McLean, 1998).

RESULTS AND DISCUSSION

Some form of sample preparation is usually required before analysis due to the detection and measurement conditions of various types of laboratory equipment. Determining the moisture content of feeds can be accomplished by multiple methods (Thiex and Richardson, 2003), and in swine experiments feeds have been dried at moderate (60°C, Stein et al., 2006) and high (95°C, Goebel and Stein, 2011) temperatures. Howev-

er, obtaining a representative sample to adhere to the AOAC method 930.15 (AOAC, 2005) requiring only 2 g may be difficult. Although this may be possible for feed samples that are relatively dry and have been ground, it would be difficult after a multiple day collection of growing-finishing pigs or poultry. The times and temperatures picked for this study are similar to those we have used in the past (Kerr et al., 2009) and are not unlike those used by others (Yen et al., 2004; Stein et al., 2006; Lindemann et al., 2010).

Previous research indicate that analysis of animal excreta, currently used in nutrition and agricultural research, may be affected by drying method (Lawrence, 1971; Sistani et al., 2001). Reduced fecal N concentration has been reported due to drying cattle feces (Galup and Hobbs, 1944; Colovos et al., 1957; Bratzler and Swift, 1959), with Falvey and Woolley (1974) reporting a decrease in N losses with increasing drying temperatures. A similar effect of increasing temperature on reducing N losses has also been reported in the drying of various poultry manure when combined with soil (Giddens and Rao, 1975) where it has been indicated that the ability of microorganisms to degrade urea or uric acid to ammonia is reduced under a more rapid, high temperature drying method compared with a slower, low temperature method. Relative to GE losses, Colovos et al. (1957) reported that GE losses in cattle feces was approximately 14% if dried at 65°C compared with

Table 4. The effect of drying method on the composition of pig urine, as-is basis¹

Analysis	Drying method ²				Model ³		Contrast ⁴		
	UD	FD	OD55	OD100	SEM	<i>P</i> -value	UD vs. dry	FD vs. OD	OD55 vs. OD100
DM, %	—	4.50	4.32	3.60	0.55	0.01	—	0.03	0.01
GE, cal/g	103.1	103.7	117.7	93.3	10.8	0.46	0.89	0.89	0.12
N, %	0.80	—	—	—	—	—	—	—	—
C, %	0.98	—	—	—	—	—	—	—	—
S, %	0.78	—	—	—	—	—	—	—	—

¹Fresh urine collected from growing pigs with 12 observations per drying method.

²Drying methods consisted of undried (UD), freeze drying (FD), and oven drying at 55°C (OD55) or 100°C (OD100).

³Model statistics.

⁴Preplanned contrast statements.

Table 5. The effect of drying method on the composition of poultry excreta, as-is basis¹

Analyses	Drying method ²				Model ³		Contrast ⁴		
	UD	FD	OD55	OD100	SEM	<i>P</i> -value	UD vs. dry	FD vs. OD	OD55 vs. OD100
DM, %	—	20.95	20.83	20.78	0.61	0.98	—	0.87	0.96
GE, cal/g	854	809	796	812	20	0.22	0.05	0.86	0.59
N, %	1.16	1.07	1.05	1.01	0.05	0.25	0.07	0.50	0.58
C, %	8.45	8.33	8.37	8.24	0.36	0.98	0.74	0.95	0.81
S, %	2.94	1.53	0.55	0.88	0.38	0.09	0.01	0.09	0.54

¹Fresh excreta collected from growing broilers with 32 observations per drying method.

²Drying methods consisted of undried (UD), freeze drying (FD), and oven drying at 55°C (OD55) or 100°C (OD100).

³Model statistics.

⁴Preplanned contrast statements.

UD feces, whereas Bratzler and Swift (1959) reported no loss of GE due to drying cattle feces at 65°C.

Consequently, if the drying method has an effect on sample composition, the accuracy of nutrient balance is reduced, making interpretation of the results biased relative to the degree of nutrient loss. In the current experiment, we chose to feed diets ranging in CP (15.06 to 18.76%) and NDF (4.5 to 15.4%) to generate feces and urine of varying DM, GE, N, S, and C composition (Kerr and Easter, 1995; Canh et al., 1997).

Drying Method: Pig Feces

The current experiment measured the impact of 4 commonly used drying methods on N and GE concentrations. Additionally, C and S were evaluated due to the impact of animal production on the release of these chemicals into the environment. When utilizing a protected *F*-test, there was no effect of drying method on DM, GE, N, or C concentrations in pig feces (Table 3). However, utilizing preplanned contrasts comparing UD to dried samples (OD50, OD100, and FD) there was a loss in GE ($P < 0.10$) of approximately 5%. However, there was no difference between FD and OD or between the 2 oven drying temperatures ($P > 0.10$; Table 3) for all analytes measured. Drying resulted in a loss of S of approximately 58% (model $P < 0.01$; UD vs. dry contrast, $P < 0.01$).

The impact of drying on GE or N content of feces in swine has been largely overlooked, with methods largely adapted from the cattle or poultry literature. Moisture losses may differ between different drying methods (Lawrence, 1971; Blake and Potter, 1987) and, as such, can affect subsequent analytical results. Fecal composition has been shown to be affected by drying methods, where differences in N and GE concentrations have been reported between OD and FD (Lawrence, 1971). Likewise, Mahimairaja et al. (1990) reported significant reductions in total N from pig slurry after samples were OD at 105°C, whereas FD had no effect on the total N concentration compared with UD manure. In contrast, Giddens and Rao (1975) indicated drying manure at a lower temperature (23°C for 10 d) tended to cause a

greater loss in N vs. fast drying at a high temperature (100°C for 8 h), but this was not observed in the current trial. We have no explanation for the relatively large loss of S compared with that noted for GE or N, but also have no other data with which to compare.

Drying Method: Pig Urine

Urine is analyzed in metabolism studies to measure patterns in metabolizable constituents, with GE and N being the most common components evaluated. This is important not only because N excretion routes change as diets differ in CP (Gatel and Grosjean, 1992; Kerr and Easter, 1995) and fiber (Zervas and Zijlstra, 2002; Shriver et al., 2003), but also due to compounds that can acidify the urine (Canh et al., 1998). In addition, ammonia is released during the conversion of urinary urea to ammonia due to microbial urease present in feces (Canh et al., 1997; van Kempen et al., 2003). Therefore, nutrient balance experiments typically involve acidification of the urine to prevent loss of N due to microbial growth (Pedersen et al., 2007). Consequently, we chose to measure the impact of urine acidification along with drying method on urine composition. In the current study, the addition of 1.5 mL of 6 M HCl to 50 mL of urine to lower the pH to 2.0 had no impact on urinary DM, GE, N, C, or S concentrations (data not shown), indicating that losses of these components were not affected by diet acidification during sample processing. These data do not, however, reflect the potential losses that would occur from the time of collection until they reach the laboratory (van Kempen et al., 2003). The current data are supported by Pan et al. (2009) who reported no impact of acidifying poultry excreta on total N compared with fresh excreta, indicating that once in the laboratory and properly stored, little N is lost during the course of laboratory procedures. In contrast, Ribeiro et al. (2001) reported a 10% loss in the N content of broiler excreta when it was not acidified to pH 4.5 before OD at 55 or 100°C. In the current experiment, there were no differences in urinary GE due to drying methods (Table 4). Because the equipment used in our laboratory analyzed urine by an UD process

only, no comparison due to drying methods is possible for N, C, or S. The apparent differences in DM between FD and OD ($P < 0.05$) and between OD55 and OD100 ($P < 0.01$) indicates that FD was more efficient in dehydrating the samples compared with the OD methods, and that OD55 was more efficient than OD100. We cannot explain these differences, but speculate that it may involve volatile compound losses under the OD conditions. Only 1 scientific report could be found that evaluated the impact of drying method on urine energy; in that instance, similar to the current experiment, FD or OD of urine had little to no effect on urinary energy concentration (Hartfiel, 1961).

Drying Method: Chicken Excreta

For poultry excreta, the use of an overall model F -test indicated there were no differences among drying methods for DM, GE, N, C, and S concentrations ($P > 0.10$; Table 5). However, the preplanned contrasts indicate that GE ($P < 0.05$), N ($P < 0.10$), and S ($P < 0.01$) were reduced by drying (FD, OD55, and OD100) by 6, 10, and 66%, respectively, compared with UD. There was no difference between FD and OD for GE or N concentration ($P > 0.10$). However, OD resulted in a decreased concentration of S compared with FD ($P < 0.10$).

Manoukas et al. (1964), Shannon and Brown (1969), and Mahimairaja et al. (1990) reported that OD resulted in greater N and GE losses compared with FD, and in some cases, FD was equal to UD. Relative to drying temperature, Shannon and Brown (1969) reported that increasing the OD temperature decreased GE losses, but increased N losses. Ribeiro et al. (2001) also reported that increasing oven temperature increased N losses. In contrast, Wallis and Balnave (1983) reported that FD had a greater impact than OD on the losses N or GE. Blake and Potter (1987) reported that increasing drying temperature increased DM percentage, but when data were converted to a fresh weight basis, there was no apparent effect on N or GE losses up to 100 to 120°C. However, above 120°C, both N and GE content were affected. Sibbald (1979) and Dale et al. (1985) reported no difference in true ME between FD or OD excreta. Finally, data reported by Sistani et al. (2001) support our findings in that FD, OD65, and OD105 increased N losses compared with UD, but there were no differences among any of the drying methods.

In the current experiment, drying method had no impact on C losses, but the greater S losses warrant further investigation because this could have dramatic impacts on interpreting manure composition and gas emission data. The current data, along with results from published literature, indicate that regardless of drying method, some loss of GE and N appears to be inevitable. However, there appear to be no distinct advantage between FD and OD relative to GE or N losses.

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