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Lack of interactive effects between diet composition or acid addition with drying method on amino acid digestibility values in porcine ileal digesta

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Abstract

Two experiments were conducted to determine the effect of oven drying (OD) or freeze drying (FD) on apparent ileal digestibility (AID) of amino acids (AA) in diets fed to pigs. In Exp. 1, 15 barrows (88.4 ± 6.4 kg) were allotted to either a corn starch-soybean meal (CST), potato starch-soybean meal (PST), or corn-soybean meal (CSBM) diet, over 2 collection periods. Following collection, samples were pooled within pig and subdivided into either OD or FD, resulting in 10 observations per diet by drying method combination. In Exp. 2, 11 barrows (63.3 ± 3.8 kg) were fed a CST diet and following collection, samples were pooled within pig and subdivided and either adjusted to pH 4 or remain unadjusted. Subsets of these samples were then subdivided to be either FD or OD, resulting in 11 observations per pH level by drying method combination. Oven drying was accomplished by drying samples in a forced air oven at either 100°C (Exp. 1) or 75°C (Exp. 2). In Exp. 1, there were no diet-type by drying-method interactions noted for any of the AA ($P > 0.10$). Oven drying resulted in a higher AID of AA compared to samples which were FD ($P \leq 0.10$), for all AA except for Gly and Tyr. Averaged across all AA, AID of AA was 3.3% greater if the sample was OD compared to FD. Differences in AID of AA among the 3 diets was noted for all AA ($P \leq 0.07$), except for Cys ($P = 0.33$), due to the fact that CST and PST diets only contained soybean meal (SBM) as an AA-providing ingredient while the CSBM diet contained both corn and SBM. Pigs fed the PST diet had greater SID for all AA compared to pigs fed the CST diet ($P \leq 0.05$), except for His, Lys, Cys, and Glu. In Exp. 2, there were no pH-adjustment by drying-method interactions noted on AID for any of the AA ($P > 0.10$). Adjusting ileal digesta to a pH of 4.0 had little effect on AID for most of the AA, except for a lowered AID of Arg, His, Lys, Trp, and Ser ($P \leq 0.10$). Oven drying resulted in a higher AID for all AA ($P \leq 0.09$) except for Ile, Thr, Val, Ala, Asp, Glu, and Gly. Averaged across all AA, the increase in AID of AA was 1.7% greater if the sample was OD compared to FD. On average, OD of ileal digesta resulted in 2.5% greater estimate of AID of AA compared to samples that were FD, and was not diet-, pH-, or AA-dependent. Because the majority of the data on AA digestibility are based on FD, a bias factor may be necessary to adjust AA digestibility data obtained on an OD-basis relative to a FD-basis for use in feed formulation.

Keywords

amino acids, freeze drying, ileal digestibility, oven drying, pigs

Disciplines

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Comments

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Lack of interactive effects between diet composition or acid addition with drying method on amino acid digestibility values in porcine ileal digesta[1]

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ABSTRACT: Two experiments were conducted to determine the effect of oven drying (OD) or freeze drying (FD) on apparent ileal digestibility (AID) of amino acids (AA) in diets fed to pigs. In Exp. 1, 15 barrows (88.4 ± 6.4 kg) were allotted to either a corn starch-soybean meal (CST), potato starch-soybean meal (PST), or corn-soybean meal (CSBM) diet, over 2 collection periods. Following collection, samples were pooled within pig and subdivided into either OD or FD, resulting in 10 observations per diet by drying method combination. In Exp. 2, 11 barrows (63.3 ± 3.8 kg) were fed a CST diet and following collection, samples were pooled within pig and subdivided and either adjusted to pH 4 or remain unadjusted. Subsets of these samples were then subdivided to be either FD or OD, resulting in 11 observations per pH level by drying method combination. Oven drying was accomplished by drying samples in a forced air oven at either 100°C (Exp. 1) or 75°C (Exp. 2). In Exp. 1, there were no diet-type by drying-method interactions noted for any of the AA ($P > 0.10$). Oven drying resulted in a higher AID of AA compared to samples which were FD ($P \le 0.10$), for all AA except for Gly and Tyr. Averaged across all AA, AID of AA was 3.3% greater if the sample was OD compared to FD. Differences in AID of AA among the 3 diets was noted for all AA ($P \le 0.07$), except for Cys ($P = 0.33$), due to the fact that CST and PST diets only contained soybean meal (SBM) as an AA-providing ingredient while the CSBM diet contained both corn and SBM. Pigs fed the PST diet had greater SID for all AA compared to pigs fed the CST diet ($P \le 0.05$), except for His, Lys, Cys, and Glu. In Exp. 2, there were no pH-adjustment by drying-method interactions noted on AID for any of the AA ($P > 0.10$). Adjusting ileal digesta to a pH of 4.0 had little effect on AID for most of the AA, except for a lowered AID of Arg, His, Lys, Trp, and Ser ($P \le 0.10$). Oven drying resulted in a higher AID for all AA ($P \le 0.09$) except for Ile, Thr, Val, Ala, Asp, Glu, and Gly. Averaged across all AA, the increase in AID of AA was 1.7% greater if the sample was OD compared to FD. On average, OD of ileal digesta resulted in 2.5% greater estimate of AID of AA compared to samples that were FD, and was not diet-, pH-, or AA-dependent. Because the majority of the data on AA digestibility are based on FD, a bias factor may be necessary to adjust AA digestibility data obtained on an OD-basis relative to a FD-basis for use in feed formulation.

Key words: amino acids, freeze drying, ileal digestibility, oven drying, pigs

List of Abbreviations: AA, amino acids; AID, apparent ileal digestible; BW, body weight; CSBM, corn-soybean meal; CST, corn starch-soybean meal; CP, crude protein; DM, dry matter; Ala, alanine; Arg, arginine; Asp, aspartic acid; Cys, cysteine; FD, freeze drying; Glu, glutamic acid; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; OD, oven drying; Phe, phenylalanine; PST, potato starch-soybean meal; SBM, soybean meal; SEM, standard error of the mean; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; Val, valine

INTRODUCTION

Accurate determination of nutrients and energy in digesta is critical in digestibility studies. While energy and N concentrations in feces, urine, or excreta has received much attention in poultry and swine nutrition studies (Jacobs et al., 2011), information on different drying methods used for ileal digesta processing prior to amino acid (**AA**) determination is limited. The idea that freeze drying (**FD**) is the preferred method for processing ileal digesta prior to AA analysis (Wallis and Balnave, 1983; Moughan et al., 1987) was based on data indicating that oven drying (**OD**) of excreta resulted in loss of N from excreta compared to FD (Manoukas et al., 1964; Shannon and Brown, 1969). Yet, the loss of energy or N from excreta as affected by drying method has been shown to be inconsistent (Jacobs et al., 2011) and the observation that loss of N from excreta does not directly imply that AA have been damaged or converted into non-AA compounds in the digesta, thereby affecting AA digestibility. Losses of N due to different drying methods could simply be related to the loss of ammonia in the digesta (Weber and Veach, 1979; Bergen and Wu, 2009; Columbus et al., 2015) that occurs during the drying process. In fact, early data comparing OD to FD of ileal digesta were inconclusive as to affecting AA concentrations (Wallis and Balnave, 1983) or AA digestibility (Dale et al., 1985). In a similar conflicting outcome, Olojede et al. (2018) reported no difference between OD and FD of ileal digesta on apparent AA digestibility in broilers, while Lagos and Stein (2019) reported that ileal digesta in growing pigs that was OD resulted in greater standardized ileal AA digestibility coefficients compared to ileal digesta that was FD. Although the majority of literature reports AA digestibility based on ileal digesta that had been FD (Huang et al., 2005; Stein et al., 2006; Widyaratne and Zijlstra, 2007; Rochell et al., 2012; Kong and Adeola, 2013; Curry et al., 2014), in some cases ileal samples have been OD (Pousga et al., 2007; Wang et al., 2008; Kerr et al., 2019), or both OD and FD (Engster et al., 1985; Ravindran et al., 1999). Consequently, additional research is needed to clarify the effect of drying method (OD versus FD) on AA digestibility and to determine if an effect is diet dependent or if samples should be pH-adjusted prior to processing to prevent potential microbial growth.

MATERIALS AND METHODS

Each experiment was reviewed and approved by the Institute Animal Care and Use Committee at Iowa State University, Ames, IA (Exp. 1, # 5-15-8022-S; Exp. 2, # 4-18-8744-S).

Animals, Housing, and Experimental Design

Fifteen (15) barrows (BW of 88 ± 6.4 kg) were used in Exp. 1 and 11 barrows (body weight, **BW**, of 63.3 ± 3.8 kg) were used in Exp. 2; whereupon each pig had a cannula surgically installed at the distal ileum to allow for collection of ileal digesta (Stein et al., 1998). Following surgery, pigs were individually housed in a temperature-controlled room in a 1.2 m × 1.5 m pen with slatted concrete sides and a partially slatted floor with a self-feeder and nipple drinker. In Exp. 1, there were 3 dietary treatments allotted to pigs over 2 collection periods, but allotted such that pigs were not assigned to the same diet for both collection periods. After collection, the ileal samples were subdivided and either OD or FD, resulting in 10 observations per diet by drying method combination (Figure 1). In Exp. 2, all pigs received only one diet, after which the ileal samples were subdivided and either pH adjusted or not, and then either OD or FD, resulting in 11 observations per pH level by drying method combination (Figure 2).

Ingredients, Diets, and Feeding

Diets in Exp. 1 consisted of a corn starch-soybean meal (**CST**), a potato starch-soybean meal (**PST**), or a corn-soybean meal (**CSBM**) diet, each of which were formulated to be adequate in vitamins and trace minerals according to the (NRC, 2012). Table 1. Titanium dioxide was included in all diets at 0.5% as an indigestible marker. The CST diet represented a diet commonly used to determine AA digestibility of ingredients; the PST diet represented a diet containing resistant starch, which if reaching the terminal ileum may form products of the Maillard reaction if reducing sugars and AA are present during a drying process, and the CSBM diet was included as a complete diet typically fed to growing pigs. In Exp. 2, all pigs were fed a CST diet formulated the same as in Exp. 1, Table 1.

Pigs were weighed at the beginning of each experiment to calculate feed allowance, which was set at 95% ad libitum, and fed in 2 equal meals per day. Diets were fed in meal form and water was free access for the entire experiment. Each period lasted 7-d, with 5-d of diet adaptation followed by 2-d ileal digesta collection for 8 h each day following the morning feeding. In each experiment, ileal digesta was collected by attaching a plastic bag to the T-cannula via a spring clamp. Bags were removed once full or every 30 min and immediately placed at -20°C. At the end of the collection period, bags were thawed and pooled within pig. In Exp. 1, pooled samples were mixed thoroughly and divided into 2 sub-samples that were either FD or OD (Figure 1). In Exp. 2, within-pig pooled samples were mixed thoroughly and divided into 2 sub-samples that were either adjusted to pH 4.0 using 6 N HCL, or remained unadjusted. These two subsets of samples were then further divided into 2 sub-samples that were either FD or OD resulting in (Figure 2).

Ileal Drying Methods

To FD samples in Exp. 1 and 2, the samples were lyophilized at -80°C for 24 h and then placed in vacuum tubes which were cooled to -110°C under a vacuum pressure of ≤ 100 μm for approximately 48 h (CS3 system with VC01 vacuum controller and CT110 cold trap; ATR Laurel, MD). Prior to OD, the forced-air oven temperature and air speed distribution (SMO 28-2; Sheldon Mfg., Cornelius, OR) was examined for consistency of air flow and temperature as shown in Figure 3. Because oven temperature and air flow in the oven was slightly irregular, samples to be dried were placed in the oven so diets were evenly distributed in the oven (e.g., front-to-back and top-to-bottom). To OD in Exp. 1, approximately 200 mL of ileal digesta was poured into aluminum pans (20 cm circumference) with 1 pan per pig. For Exp. 1 the oven temperature was set at 100°C and samples were dried for approximately 13 h. For Exp. 2, samples were similarly placed in aluminum pans and dried for approximately 13 h, but with an oven temperature of 75°C. Following FD and OD, diets and ileal digesta were finely ground through a 1-mm screen in preparation for chemical composition determination. Samples were analyzed for DM (AOAC 930.15, AOAC, 2007), TiO_2 (Leone, 1973), and AA (AOAC 994.12, AOAC, 2007; ISO 13904, ISO, 2016).

Calculation and Statistical Analysis

Analyzed TiO_2 and AA levels of each diet and ileal digesta sample were used to calculate apparent ileal digestibility of AA in each diet using indirect marker methodology. All data were subjected to ANOVA using the general linear model procedures of SAS (SAS 9.4, Inc., Cary, NC) as appropriate for a factorial arrangement of treatments. In Exp. 1, treatments consisted of drying method (OD and FD) and diet type (CST, PST, and CSBM) as the main factors, with group retained in the model. In Exp. 2, treatments consisted of with drying method (OD and FD) and pH adjustment (no and yes) as the main factors. In Exp. 1 and Exp. 2, there were no interactions noted ($P > 0.10$) between the main effects, such that the interaction term was removed from the model and the model re-run. Where necessary, differences between treatment means were compared using LSMEANS, with the level of significance set at $P \leq 0.10$.

RESULTS AND DISCUSSION

In Exp. 1, there were no diet-type by drying-method interactions noted for any of the AA measured ($P > 0.10$), despite the fact that ileal digesta from pigs fed the PST diet contained 62.4% starch compared to 3.2% or 5.6% starch in ileal digesta from pigs fed the CST and CSBM diets, respectively; Table 2. It was hypothesized that a diet high in resistant starch (i.e., PST) would have provided some reducing sugars in the terminal ileum which, when present with undigested AA and subsequently oven dried, would result in Maillard reactions occurring, and therefore, differentially affect AA digestibility (Pahm et al., 2008, Gonzalez-Vega, 2011). The data, however, suggest no apparent Maillard-type reaction appeared to occur as supported by no diet by drying method interaction. Our results are supported by Lagos and Stein (2019) who did not report an interaction between diet type and AA digestibility when the ileal digests was either FD or OD; where diets containing 20% lactose fed to 14 kg pigs or diets containing DDGS fed to 73 kg pigs were evaluated in an attempt to provide reducing sugars at the terminal ileum. The lack of an interaction in Exp. 1 in addition to the data reported by Lagos and Setin (2019) suggests what little, if any Maillard-type reactions occurs due to oven drying of ileal digesta, at least in terms the diets tested and the OD methods evaluated.

In Exp. 1, the method of ileal drying, OD versus FD, affected the digestibility ($P \leq 0.10$) of all AA except for Gly and Tyr, where it was observed that samples which were OD resulted in a higher AA digestibility compared to samples which were FD, Table 2. On average, AA digestibility was 3.3% greater if the sample was OD compared to FD, but the relative increase in digestibility did not differ greatly among the AA measured, with the digestibility of AA due to OD being 1.0% to 5.7% greater than the digestibility of AA due to FD. This difference in AA digestibility was not significant among the AA as noted by the lack of an interaction between diet and drying method for any AA analyzed. This data is supported by Lagos and Stein (2019) who reported that AA digestibility due to OD samples was on average 5.4% or 7.8% greater compared to FD for 14 kg pigs fed a corn starch-lactose-soybean meal based diet or for 73 kg pigs fed a corn starch-DDGS based diet, respectively. Even though Dale et al. (1985) reported increased Arg, Lys, Met, and Tyr digestibility in broiler excreta that was OD compared to FD, differences in digestibility for all other AA were not found to be significant, and when averaged across all AA, the difference in AA digestibility whether the sample was OD or FD averaged only 0.3% greater for samples that had been OD. In contrast, Olojede et al. (2018) reported no differences in AA digestibility of boilers fed a corn-soybean meal-barley based diet between samples that were either OD or FD, while Wallis and Balnave (1983) reported greater concentrations of AA in samples that were OD versus samples that were FD (i.e., an average increase of 4.5%) which would imply that AA digestibility would have been lower in OD samples compared to samples that had been FD.

The differences in AA digestibility among the 3 diets noted for all AA ($P \leq 0.07$), except for Cys ($P = 0.33$), Table 2, were expected because pigs fed the CST and PST diets only contained soybean meal (**SBM**) as an AA-providing ingredient, while pigs fed the CSBM diet contained both corn and SBM as AA-providing ingredients. It was not expected, however, that AA digestibilities would differ between pigs fed the CST and PST diets, where it was observed that AA digestibilities were higher for all AA in pigs fed the PST diet compared to pigs fed the CST diet ($P \leq 0.05$), except for His, Lys, Cys, and Glu whose digestibilities were not found to be different. We have no explanation for this apparent difference and could not find data in the literature to compare with.

In Exp. 2, there were no pH-adjustment by drying-method interactions noted for any of the AA measured ($P > 0.10$). Even though bacterial growth is optimal at a neutral pH, microbial growth can occur between a pH of 4.5 and 9.5 (Lambert, 2010; Akkermans and Van Impe, 2018). Consequently, it was decided to test the effect of reducing the pH of the ileal digesta to 4.0 in an attempt to greatly reduce or prevent microbial growth in the ileal sample which might occur during a subsequent OD process. In general (Lapalce et al., 1985; Clark et al., 1992; Sok et al., 2017) microbial protein has the greatest concentrations of Glu (12.3% of total AA), Asp (11.7%), and Leu (8.3%), and the lowest concentrations of Cys (1.3%), His (2.1%), and Met (2.4%). Therefore, if samples in Exp. 1 or 2 had been contaminated with bacterial growth during the OD process, the digestibilities of Glu, Asp, and Leu would have decreased relative to the digestibilities of Cys, His, and Met, which would have increased. While this numerically occurred in Exp. 1 (Glu, Asp, and Leu; OD vs FD = 102.5% versus Cys, His, and Met, OD vs FD = 103.8%) and in Exp. 2 (Glu, Asp, and Leu; OD vs FD = 100.9% versus Cys, His, and Met, OD vs FD = 102.2%), the average difference across the two experiments is small (OD vs FD = 101.3%) and difficult to test experimentally as well as to determine if the differences are biologically relevant. Likewise, sample pH adjustment to pH 4.0 in Exp. 2 to prevent or greatly reduce bacterial growth should have essentially increased Glu, Asp, and Leu digestibility relative digestibilities of Cys, His, and Met, but this did not occur (Glu, Asp, and Leu; no acid vs pH adjusted = 100.5%; Cys, His, and Met, no acid vs pH adjusted = 100.9%). Taken together, the data reported in Exp. 1 and 2 suggest that microbial growth did not occur relative to the OD process and that pre-treatment with an acid to lower pH to prevent microbial growth is not necessary.

Aside from potentially affecting microbial growth and subsequent AA digestibilities, adjusting ileal digesta to a pH of 4.0 in Exp. 2 had little effect on digestibility for most of the AA evaluated, Table 3, except for a lowered ($P \leq 0.10$) digestibility of Arg, His, Lys, Trp, and Ser. It is worthy to note that Arg, His, and Lys are all basic-AA, but no data could be found suggesting that digestibility of these AA would be affected by pretreating ileal digesta to a pH of 4.0 using HCl. This is not surprising given that proteins are digested using 6N HCl prior to AA determination. The reduction in Trp digestibility due to lowering pH to 4.0 was not unexpected given that proteins are alkaline-digested using 4.2N NaOH to determine Trp concentrations in feedstuffs, because it is well known that acid-digestions destroy Trp. In addition, a reduction in Ser was not unforeseen given that Ser is commonly regarded as an acid-labile AA (Rowan et al., 1992; Darragh et al., 1996). While Met and Cys are also acid-labile AA (prior to acid hydrolysis Met and Cys are oxidized to methionine sulfone and cysteic acid, respectively), they were not affected ($P \geq 0.12$) by pH adjustment to 4.0 in the current study. In addition, they were affected in a different direction where Met digestibility was numerically increased ($P = 0.12$) and Cys digestibility was numerically decreased ($P = 0.17$).

In Exp. 2, drying method (e.g., OD versus FD) affected AA digestibility ($P \leq 0.09$) for all AA except for Ile, Thr, Val, Ala, Asp, Glu, and Gly, where it was observed that samples which were OD had a higher AA digestibility compared to samples which were FD, Table 3. Averaged across all AA, the increase in AA digestibility was 1.7% greater if the sample was OD compared to FD. Similar to Exp. 1, the increase in digestibility did not differ greatly among the AA measured, where AA digestibility due to OD was 0.4% to 5.5% greater than AA digestibility determined using FD. The increased AA digestibility due to OD in Exp. 2 is supportive of the data in Exp. 1, but the increase in AA digestibility were smaller (3.3% in Exp. 1 and 1.7% in Exp. 2), which may be due to the lower drying temperature in Exp. 2 (75°C) compared to Exp. 1 (100°C). Wallis and Balnave (1983) reported that drying poultry excreta at 80°C generally lowered AA concentrations compared to drying excreta at 60°C, which would have resulted in increased AA digestibility coefficients, similar to the data reported herein. In contrast, Olojede et al. (2018) reported no significant difference in AA digestibility in ileal digesta of broilers if the digesta was OD at 40°C compared to 55°C.

It is worthy to note that in Exp. 1 and 2, only 13 h was needed to dry samples at either 100°C (Exp. 1) or 75°C (Exp. 2), which was due to the small amount of sample being dried (200 mL), samples were dried using a container with a large drying surface area (314 cm^2 aluminum pans) relative to the amount of sample being dried, and a force-air oven which had a relative moderate movement of air (0.15 m/sec). Others (Wallis and Balnave, 1983, 60°C or 80°C for 24 h; Dale et al., 1985, 60°C for 18 h; Olojede et al., 2018, 40°C or 55°C for 144 h; Lagos and Stein, 2019, 65°C for 144 h) also used force air ovens to OD their samples, however, no information was provided on the volume of sample dried, the drying surface area, or the air speed; all of which could affect the rate of drying and any subsequent microbial growth or as potential over-heating of the sample. The use of 75°C and 100°C for OD in the current experiment was based on using 100°C in a previous drying-method experiment (Jacobs et al., 2011) and 70°C in previous metabolism studies (Kerr et al., 2013, 2017) as well as to have the OD samples be dried rapidly because time and temperature are known to impact on microbial growth (Ratkowsky et al., 1982).

While many have evaluated how OD and FD affects nitrogen and energy concentrations in ileal digesta or fecal matter (Manoukas et al., 1964; Shannon and Brown, 1969; Jorgensen et al., 1984; Dale et al., 1985; Sibblad, 1979; Mahimairaja et al., 1990; Riberio et al., 2001; Jacobs et al., 2011; Olojede et al., 2018; Lagos and Stein, 2019), the observation that method of drying affects nitrogen digestibility does not directly imply that OD damages or coverts AA into non-AA compounds in the digesta, which would consequently affect AA digestibility. Differences in nitrogen concentrations, losses, or digestibility due to different drying methods could simply be related to the loss of ammonia in the digesta (Weber and Veach, 1979; Bergen and Wu, 2009; Columbus et al.,

2015) that may occur during the drying process. If it is assumed that AA are destroyed or converted to other non-AA compounds, one might speculate that certain AA would be more susceptible to damage compared to other AA. If this is the case, the relative change in AA digestibility should be higher, or lower, depending upon the specific AA. In Exp. 1, the greatest increase in digestibility due to OD compared to FD occurred for Gly, His, and Thr; and the least for Tyr and Glu. In Exp. 2, the greatest increase in digestibility due to OD compared to FD occurred for Lys and Gly; and least for Asp, Val, and Thr. In data reported by Lagos and Stein (2019), the greatest increase in digestibility due to OD compared to FD occurred for Cys, Lys, and Glu, and least for Ala and Ile in Exp 1, while in Exp. 2 the greatest increase in digestibility due to OD compared to FD occurred for Lys, Asp, Cys, and His; and least for Trp, Ala, and Gly. Because there is no consistency on which AA were affected among these two research locations or between experiments within each location, it appears that the increased AA digestibility relative to OD versus FD is a general effect on all AA and not on a specific AA or class of AA. As to why OD versus FD seems to affect the digestibility coefficients of AA in swine ileal digesta (Exp. 1 and 2; Logos and Stein, 2019) and not in poultry excreta (Wallis and Balnave, 1983; Dale et al., 1985; Olojede et al., 2018) needs further experimentation.

CONCLUSION

Oven drying of ileal digesta resulted in greater estimates of apparent ileal digestibility of AA, independent of diet, pH, and AA. The data suggest that at the OD temperatures evaluated, there was no growth of microbes during the drying process that affected AA digestibility and that no adjustment to pH prior to processing was needed to prevent microbial growth. The difference in AA digestibility between samples that were OD versus FD suggests that AA were damaged or converted to other non-AA compounds. Because the majority of the research published on determining AA digestibility coefficients are based on FD samples, a bias factor may be necessary to adjust AA digestibility data obtained from OD samples versus FD samples for comparative purposes and for use in feed formulation.

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Figure 1. Sample flow for processing of ileal digesta, Exp. 1. CST = corn starch-soybean diet, PST = potato starch-soybean meal diet, CSBM = corn-soybean meal die

Figure 2. Sample flow for processing of ileal digesta, Exp. 2.

Figure 3. Air speed and dry-bulb temperature spatial distribution of forced–air oven used in Exp. 1 and 2. Shelf: A is located at the bottom of the oven.

Table 1. Ingredient and analyzed composition (%) of experimental diets, as-is basis, Exp. 1 and Exp. 2

	Exp. 1			Exp. 2
Item	Corn starch	Potato starch	Corn-soybean meal	Corn starch
Corn	-	-	74.25	-
Soybean meal	30.00	30.00	20.75	30.00
Corn starch	45.00	-	-	45.00
Potato starch	-	45.00	-	-
Sucrose	20.00	20.00	-	20.00
Soybean oil	2.00	2.00	2.00	2.00
Limestone	-	-	1.00	-
Cellulose	1.75	1.75	-	1.75
Monocalium phosphate	-	-	0.65	-
NaCl	0.35	0.35	0.35	0.35
Trace mineral mix[1]	0.20	0.20	0.20	0.20
Vitamin mix[2]	0.20	0.20	0.20	0.20
L-Lys·HCl	-	-	0.10	-
Titanium dioxide	0.50	0.50	0.50	0.50
TOTAL	100.00	100.00	100.00	100.00
Analyzed composition				
DM	91.81	90.28	88.88	90.75
CP	14.74	15.14	15.73	14.75
Ala	0.61	0.68	0.81	0.75
Arg	0.99	1.07	0.93	1.19
Asp	1.59	1.75	1.50	1.94
Cys	0.19	0.20	0.24	0.23
Glu	2.54	2.75	2.64	3.11
Gly	0.59	0.65	0.61	0.73
His	0.35	0.38	0.37	0.46

Ile	0.62	0.73	0.66	0.82
Leu	1.08	1.22	1.41	1.32
Lys	0.91	0.97	0.85	1.08
Met	0.19	0.21	0.23	0.23
Phe	0.75	0.80	0.78	0.87
Ser	0.73	0.78	0.75	0.74
Thr	0.54	0.59	0.56	0.66
Trp	0.17	0.20	0.17	0.20
Tyr	0.39	0.44	0.44	0.54
Val	0.63	0.75	0.74	0.87

[1]Provided the following per kilogram of diet: 22 mg Cu (as $CuSO_4$), 220 mg Fe (as $FeSO_4$), 0.4 mg I (as $Ca(IO_3)_2$), 52 mg Mn (as $MnSO_4$), 220 mg Zn (as $ZnSO_4$), and 0.4 mg Se (Na_2SeO_3).

[2]Provided the following per kilogram of diet: 6,125 IU vitamin A, 700 IU vitamin D_3, 50 IU vitamin E, 30 mg vitamin K, 0.05 mg vitamin B_{12}, 11 mg riboflavin, 56 mg niacin, and 27 mg pantothenic acid.

Table 2. Apparent ileal amino acid digestibility of pigs fed corn starch-soybean meal (CST), potato starch-soybean meal (PST), or corn-soybean meal (CSBM) diets as affected by ileal digesta that was either freeze-dried or oven-dried, DM basis, Exp. 1

	Diet[1]					Drying method[2]			
AID, %[3]	CST	PST	CSBM	SEM	*P-value*	Freeze	Oven	SEM	*P-value*
Indispensable									
Arg	87.4[b]	91.9[a]	88.9[b]	0.65	0.01	87.9	90.9	0.53	0.01
His	88.4[a]	89.1[a]	86.5[b]	0.54	0.01	85.8	90.2	0.43	0.01
Ile	80.3[b]	86.3[a]	79.5[b]	0.70	0.01	80.9	83.1	0.57	0.01
Leu	81.8[b]	86.2[a]	82.9[b]	0.65	0.01	82.4	84.9	0.52	0.01
Lys	88.9[a]	89.2[a]	86.2[b]	0.52	0.01	86.6	89.6	0.42	0.01
Met	85.3[b]	87.7[a]	84.8[b]	0.66	0.01	84.6	87.0	0.54	0.01
Phe	84.5[b]	87.2[a]	82.4[c]	0.57	0.01	83.6	85.8	0.46	0.01
Thr	77.7[b]	80.9[a]	72.1[c]	0.92	0.01	75.2	78.7	0.75	0.01
Trp	78.2[b]	83.2[a]	70.7[c]	0.79	0.01	76.2	78.5	0.64	0.01
Val	78.4[b]	84.6[a]	77.3[b]	0.76	0.01	78.9	81.3	0.61	0.01
Dispensable									
Ala	72.7[b]	78.9[a]	76.2[a]	1.06	0.01	74.8	77.2	0.86	0.05
Asp	82.8[b]	85.8[a]	80.0[c]	0.66	0.01	81.8	83.9	0.53	0.01
Cys	72.1	69.8	69.4	1.32	0.33	69.2	71.7	1.06	0.10
Glu	85.5[ab]	87.2[a]	85.4[b]	0.61	0.07	85.3	86.8	0.49	0.03

Gly	59.6[b]	67.1[a]	63.6[ab]	2.09	0.05	61.7	65.2	1.69	0.15
Ser	82.9[b]	86.0[a]	80.0[c]	0.72	0.01	81.4	84.6	0.58	0.01
Tyr	82.5[b]	89.4[a]	81.3[b]	0.68	0.01	84.0	84.8	0.55	0.34
Starch, %	3.2[b]	62.4[a]	5.6[b]	0.96	0.01	26.0	21.5	0.78	0.01
DM, %	85.8[c]	90.3[a]	87.6[b]	0.31	0.01	88.6	87.2	0.25	0.01

[1]Represents 20 observations per diet.

[2]Represents 30 observations per drying method.

[3]AID = apparent ileal digestibility.

[abc]Means without a common superscript differ, P ≤ 0.05.

Table 3. Apparent ileal digestibility of amino acids of pigs fed a corn starch-soybean meal diet as affected by ileal digesta that was either freeze-dried or oven-dried in combination without or with a pH adjustment to 4.0, DM basis, Exp. 2

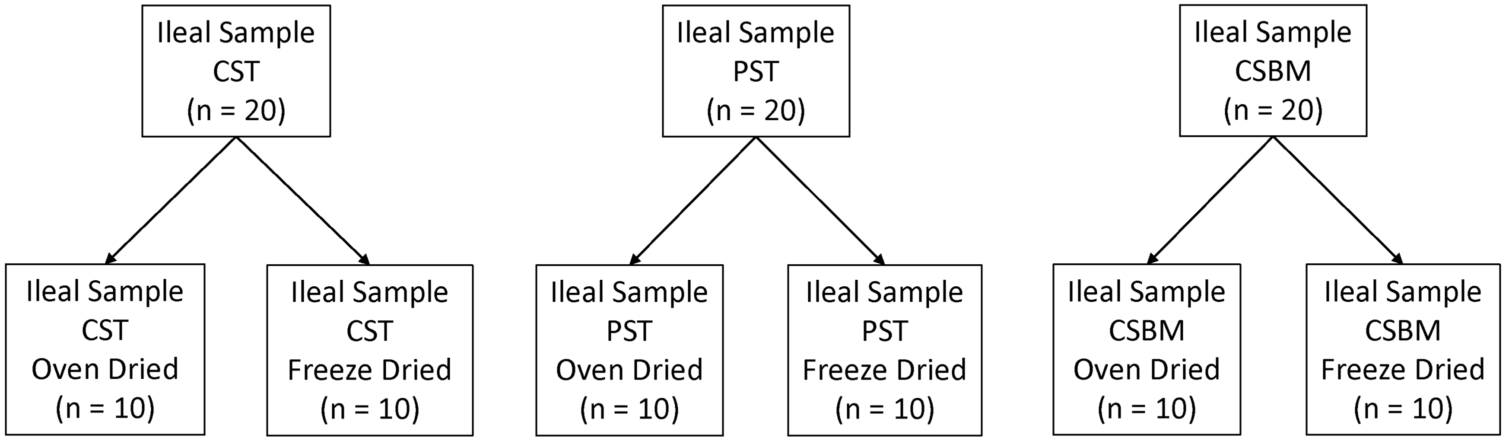
	pH adjusted to 4.0[1]				Drying method[1]			
AID, %[2]	No	Yes	SEM	P-value	Freeze	Oven	SEM	P-value
Indispensable								
Arg	91.8	89.8	0.51	0.01	89.9	91.6	0.51	0.03
His	87.7	86.1	0.40	0.01	86.0	87.8	0.40	0.01
Ile	84.6	84.5	0.37	0.88	84.3	84.8	0.37	0.35
Leu	84.9	84.3	0.37	0.26	84.2	85.1	0.37	0.07
Lys	84.4	82.7	0.60	0.05	81.3	85.8	0.60	0.01
Met	86.6	87.4	0.37	0.12	86.2	87.8	0.37	0.01
Phe	86.2	85.8	0.36	0.39	85.5	86.5	0.36	0.06
Thr	78.4	77.8	0.45	0.42	77.9	78.3	0.45	0.60
Trp	87.1	85.8	0.36	0.02	85.6	87.3	0.36	0.01
Val	80.8	80.7	0.41	0.82	80.6	80.9	0.41	0.67
Dispensable								
Ala	77.3	77.0	0.67	0.75	76.8	77.5	0.67	0.49
Asp	84.9	84.6	0.40	0.67	84.6	84.9	0.40	0.64
Cys	73.8	72.5	0.66	0.17	72.3	74.1	0.66	0.07
Glu	86.4	86.0	0.58	0.57	85.7	86.7	0.58	0.25

Gly	67.0	65.3	1.57	0.45	64.7	67.6	1.57	0.20
Ser	84.6	83.9	0.33	0.10	83.8	84.7	0.33	0.08
Try	85.4	84.9	0.40	0.38	84.7	85.7	0.40	0.09
DM, %	90.1	91.6	0.37	0.01	90.9	90.8	0.37	0.78

[1]Represents 22 observations per mean.

[2]AID = apparent ileal digestibility.

Figure 1


Ileal Sample
CST
(n = 20)
Ileal Sample
PST
(n = 20)
Ileal Sample
CSBM
(n = 20)
Ileal Sample
CST
Oven Dried
(n = 10)
Ileal Sample
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Freeze Dried
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Oven Dried
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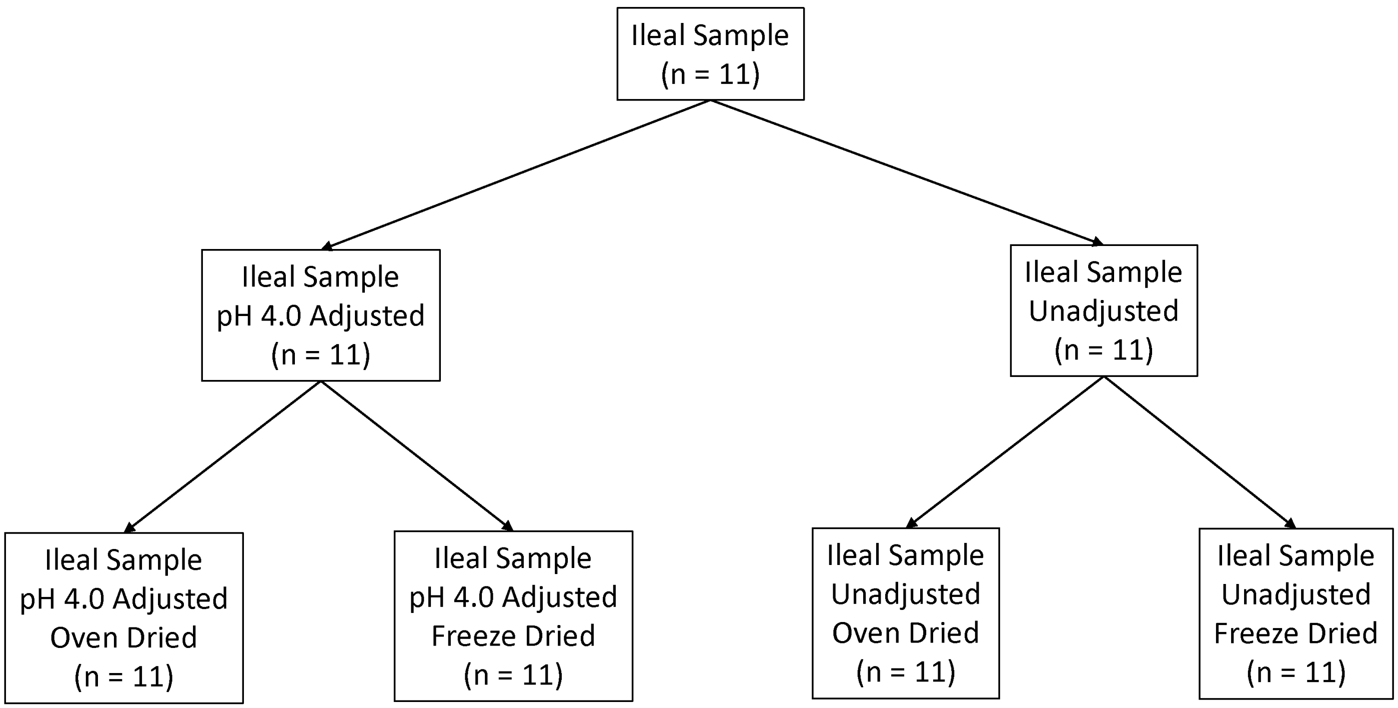
Freeze Dried
(n = 10)

Figure 2



Ileal Sample
(n = 11)
Ileal Sample
pH 4.0 Adjusted
(n = 11)
Ileal Sample
Unadjusted
(n = 11)
Ileal Sample
pH 4.0 Adjusted
Oven Dried
(n = 11)
Ileal Sample
pH 4.0 Adjusted
Freeze Dried
(n = 11)
Ileal Sample
Unadjusted
Oven Dried
(n = 11)
Ileal Sample
Unadjusted
Freeze Dried
(n = 11)

Figure 3