

Composition and variability of US soybean meal intended for swine diets

by

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Abstract

Soybean meal (SBM) is the predominant plant-based protein source used in swine diets because of its favorable amino acid profile, high nutrient digestibility, widespread availability, and relatively low cost. However, SBM contains antinutritional factors, including trypsin inhibitor, and its nutritional value may vary because of differences in soybean composition, processing conditions, and analytical methodology. Therefore, the objectives of this thesis were to evaluate the effects of increasing dietary trypsin inhibitor units (TIU) on nursery pig growth performance and nutrient digestibility, characterize the composition and variability of SBM produced by US soybean processing plants, and quantify analytical variation within and between laboratories when measuring SBM composition and variability. To address the effects of trypsin inhibitor on growth performance, 360 nursery pigs were used in a 42-d growth trial to evaluate the effects of increasing dietary TIU across six treatments ranging from 1.4 to 4.9 TIU/mg of complete feed. Soy flour (76 TIU/mg) was added at the expense of SBM (5 TIU/mg) to create experimental diets. Increasing dietary TIU decreased average daily gain, average daily feed intake, and gain:feed ratio during all three dietary phases and overall, resulting in a 5.6 kg reduction in final body weight from the lowest to greatest TIU concentration. Increasing TIU also reduced the apparent total tract digestibility of crude protein. Based on the SBM inclusion rates used in this experiment, SBM containing greater than approximately 6 TIU/mg has the potential to decrease nursery pig growth performance and nutrient digestibility. To address the chemical composition of SBM, 271 samples were collected from 29 US soybean processing plants at two timepoints within the same crop year. Samples were analyzed for nutrient composition, processing quality indicators, and physical characteristics. Soybean meal composition was relatively consistent between sampling timepoints; however, processing method

influenced several compositional and quality characteristics. Solvent extracted SBM contained greater crude protein, but lower ether extract and TIU than extruded-expelled SBM.

Considerable variation within and between processing plants was observed for ether extract, calcium, TIU, potassium hydroxide protein solubility, protein dispersibility index, and particle size, whereas crude protein, amino acid concentrations, and fatty acid profiles were comparatively consistent. In a companion study of the soybean meal composition experiment, SBM samples from 27 soybean processing plants were collected to evaluate analytical variation between and within laboratories for proximate composition, neutral detergent fiber (NDF), Ca, P, trypsin inhibitor, and amino acid profile. Samples were analyzed in quadruplicate at four laboratories. Dry matter and crude protein exhibited the lowest analytical variation and demonstrated strong repeatability within laboratories and reproducibility between laboratories. Ether extract, NDF, select amino acids, and trypsin inhibitor exhibited greater variation between laboratories. In conclusion, these studies establish the negative effects of increasing dietary TIU on nursery pig growth performance and nutrient digestibility, provide an updated benchmark of US SBM composition and variability, and demonstrate the importance of laboratory and analytical method selection when interpreting SBM quality measurements.

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Chapter 1 - Effects of increasing trypsin inhibitor on nursery pig growth performance, fecal dry matter, nutrient digestibility, and plasma cholecystokinin

Abstract

A total of 360 barrows (DNA 200 × 400; initially 6.1 ± 0.03 kg) were used in a 42-d growth trial to evaluate the effects of increasing trypsin inhibitor units (TIU) in complete feed on nursery pig growth performance, fecal dry matter (DM), nutrient digestibility, and plasma cholecystokinin. At weaning, pigs were blocked by BW and randomly assigned to pens with five pigs per pen and 12 pens per treatment. There were six dietary treatments formulated to provide 1.4, 2.1, 2.8, 3.5, 4.2, and 4.9 TIU/mg of complete feed. Soy flour (76 TIU/mg) was added at the expense of soybean meal (5 TIU/mg) to create the experimental diets. Diet formulation was based on analyzed nutrient values of the soy flour and soybean meal, assuming digestibility coefficients for soy flour were equivalent to those of soybean meal. Experimental diets were fed in three phases: phase 1 from d 0 to 10, phase 2 from d 10 to 24, and phase 3 from d 24 to 42 after weaning. Feces were collected from three pigs per pen on d 10 and 42 to determine fecal DM, and d 42 fecal samples were used to determine apparent total tract digestibility (ATTD) of DM and crude protein (CP). Across all three individual phases and overall (d 0 to 42), increasing TIU decreased (linear, $P < 0.001$) average daily gain (ADG), average daily feed intake (ADFI), and gain:feed ratio (G:F). Fecal DM on d 10 increased (linear, $P < 0.015$; quadratic, $P = 0.088$) as TIU increased, with the majority of increase in fecal DM observed as trypsin inhibitor increased from 3.5 to 4.9 TIU/mg of complete feed. However, there were no differences in fecal DM on d 42. The ATTD of DM decreased then increased (quadratic, $P = 0.003$) as TIU

increased with the lowest ATTD of DM observed at 4.2 TIU/mg of complete feed. The ATTD of CP decreased (linear, $P < 0.001$) as TIU/mg of complete feed increased. On d 10, pigs were fasted for 6 h, then allowed to consume feed for 1h prior to blood collection for plasma cholecystokinin concentrations; however, no differences were observed. In conclusion, these data suggest that increasing dietary TIU above 1.4 TIU/mg of complete feed linearly decreased nursery pig growth performance and reduced the ATTD of DM and CP. Based on the complete feed soybean meal TIU concentrations and SBM inclusion rate used in this study, soybean meal with greater than 6 TIU/mg would be above this threshold and has the potential to decrease nursery pig performance.

Key Words: antinutritional factors, soybean meal, trypsin inhibitor, weanling pigs

Introduction

Soybean meal (SBM) is the predominant plant-based protein source used in swine diets due to its favorable amino acid profile, high digestibility, and consistent supply across global markets. However, SBM contains antinutritional factors (ANF) such as trypsin inhibitor, that if not thermally processed correctly, can reduce its nutritional value, most notably for young pigs transitioning to solid feed after weaning (Liener, 1976; Vagadia et al., 2017). During this period, pigs commonly experience a transitory period of hypersensitivity to antigenic soy proteins, which can contribute to postweaning diarrhea (PWD), villus atrophy, and reduced growth performance (Li et al., 1991; Pluske et al., 1997; Lallès et al., 2004). Trypsin inhibitor decreases protein and amino acid digestibility by interfering with pancreatic protease activity of trypsin and chymotrypsin (Liener, 1976; Goebel and Stein, 2010). Trypsin inhibitors occur naturally in

soybeans as two major protein families known as Kunitz trypsin inhibitor (KTI) and Bowman-Birk inhibitor (BBI) that protect the plant from predation (Kunitz, 1946; Birk, 1985). These proteins bind with trypsin and chymotrypsin, resulting in pancreatic enzyme hypersecretion, increased endogenous amino acid losses, and reduced growth performance (Schneeman et al., 1977; Gu et al., 2010). Weanling pigs have limited digestive capacity, so even modest concentrations of TIU can elicit disproportionate reductions in protein utilization and feed efficiency (Zhang et al., 2020).

Despite improvements in commercial soy processing, a recent survey observed substantial variation in TIU concentrations among SBM sources in the United States (Gaffield et al., 2024). The results of this industry survey of over 14 soy processing plants indicated wide variations in TIU across SBM sources in the United States, with values ranging from 1.67 to 10.57 TIU/mg. This variation in soybean meal TIU has prompted the resurgence of research evaluating the acceptable levels of TIU in nursery pig diets. Nisley et al. (2025) observed that increasing dietary TIU (0.50 to 6.0 TIU/mg) through the inclusion of raw soybeans linearly decreased nursery pig ADG and G:F. Similarly, Miller et al. (2025a) reported reduced nutrient digestibility and growth performance in nursery pigs when TIU was increased (0.61 to 3.51 TIU/mg) using soy flour, and Miller et al. (2025b) observed comparable reductions in growing pigs when TIU was increased (0.99 to 9.38 TIU/mg) using raw soybeans. Collectively, these studies demonstrated that elevated dietary TIU negatively affects pig performance and additional research is needed to establish practical TIU thresholds in conventional corn-soybean meal diets. Given the considerable variation in TIU observed among commercially available SBM sources, additional research was warranted to establish practical TIU thresholds using a soy flour titration

model that allowed dietary TIU to be systematically increased while minimizing changes in ingredient composition beyond the increase in TIU itself.

Therefore, the objective of this study was to evaluate the effect of increasing TIU on nursery pig growth performance, fecal DM, nutrient digestibility, and plasma cholecystokinin. While it was hypothesized that increasing dietary TIU would decrease these criteria, it was anticipated that a maximum TIU value could be established beyond which these variables would decrease.

Materials and methods

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment (IACUC #4943). This study was conducted at the Kansas State University Segregated Early Weaning facility in Manhattan, KS. The facility has two identical barns that are completely enclosed, environmentally controlled, and mechanically ventilated. Each pen (1.2×1.2 m) contained a 4-hole, dry self-feeder and a cup waterer providing *ad libitum* access to feed and water. Facilities were mechanically ventilated with room temperatures set at 32.2°C during the first 10 d and then gradually reduced by 0.28°C daily until the set point reached 25°C. Overall pig health was excellent and only 1 pig was removed from the study, not treatment related.

Animals and diets

A total of 360 weanling barrows (DNA 200 $\times$ 400, Columbus, NE; initially 6.1 ± 0.03 kg) were used across 2 barns in a 42-d growth trial. Pigs were weaned at approximately 21 d of age and assigned to pens blocked by initial body weight (BW). Treatments were assigned in a generalized randomized block design. Pigs were blocked into light (initially 5.1 ± 0.02 kg), intermediate (initially 6.0 ± 0.02 kg), and heavy groups (initially 7.1 ± 0.02 kg). Within each

block, there were 4 pens of 5 pigs per treatment (2 pens per weight group in each barn) for a total of 12 replications per treatment (six per barn). Pens of pigs were randomly allotted to one of six dietary treatments. Diets were corn-soybean meal-based and consisted of increasing TIU/mg of complete feed (1.4, 2.1, 2.8, 3.5, 4.2, or 4.9). The lowest TIU containing diet was based on what might be considered common U.S. industry soybean meal inclusion levels for nursery pig diets. Soy flour (Prolia® FLR-100/90760, Cargill, Minneapolis, MN: 76 TIU/mg) was added at the expense of soybean meal (5.4 TIU/mg) to achieve increasing TIU levels while having the same total soy product inclusion. Dietary TIU were formulated to be identical across the three phases. Samples of soybean meal and soy flour were collected prior to diet formulation and analyzed at the University of Missouri Agricultural Experiment Station Chemical Laboratory for dry matter (Method 934.01; AOAC, 2006), crude protein (Method 990.03; AOAC, 2006), crude fat (Method 920.39; AOAC, 1998), crude fiber (Method 978.10, AOAC, 2006), ash (Method 942.05; AOAC, 2005), and complete AA profile (Method 982.30 E (a, b, c), 988.15; AOAC, 2006), and protein dispersibility index (AOCS, 2009). The soybean meal and soy flour were analyzed in triplicate for TIU at Agricultural Research Service, US Department of Agriculture (Colombia, MO) following the procedure described by Kim and Krishnan (2023; Table 1). Diets were then formulated using these analyzed nutrient values of the soy flour and soybean meal, assuming digestibility coefficients for soy flour equivalent to those of soybean meal (NRC, 2012). The net energy of soy flour was assumed to be the same as soybean meal.

Pigs were fed treatment diets in meal form for all three nursery phases with phase 1 from d 0 to 10, phase 2 from d 10 to 24, and phase 3 from d 24 to 42 (Tables 2, 3, and 4). Diets were fed in mash form to eliminate any potential TIU denaturation from the pelleting process. Treatment diets were manufactured at the Kansas State University O.H. Kruse Feed Technology

Innovation Center in Manhattan, KS. During bagging, complete diet samples were collected from every third bag using a feed probe, pooled, and stored at -20°C until analysis. Diet samples were analyzed at the Kansas State University Swine Laboratory for DM and CP, while TIU was analyzed similar to the ingredient analysis of soybean meal and soy flour (Tables 2 to 4).

Pig weights and feed disappearance were measured on d 0, 10, 17, 24, 31, and 42 to determine average daily gain (ADG), average daily feed intake (ADFI), and gain-to-feed ratio (G:F). Blood was collected on d 10 from the jugular vein of one pig, closest to the mean weight of each pen, using a lithium heparin blood collection tube for cholecystokinin (CCK) concentrations. Pigs were fasted for 6 h and then given access to feeders for 1 h prior to blood collection to ensure feed consumption. Samples were centrifuged at $1,800 \times g$ for 30 min at 4°C. Plasma aliquots were stored at -80°C until analysis by ELISA (LS Bio, Shirley, MA) using a Biotek Epoch2 microplate spectrophotometer (Santa Clara, CA).

Dry matter and digestibility calculations

Feces were collected from 3 pigs per pen on d 10 and 42 to determine percentage fecal dry matter (DM). Fecal samples were analyzed separately for each pig, and the average of the three samples from each pen was then used for statistical analysis. Fecal samples were dried at 55°C for 48 h. The loss of weight was used to calculate percentage fecal DM. Additionally, titanium dioxide (0.50%) was added in phase 3 diets as an indigestible marker to determine apparent total tract digestibility (ATTD) of DM and CP from samples collected on d 42. Following fecal DM determination, both ground feed and fecal samples were dried at 135°C for 2 h to determine percentage DM of the samples used for titanium analysis (AOAC 985.01, 2007). Titanium dioxide concentration in both dried feed and fecal samples was determined

utilizing procedures outlined by Leone (1973). The ATTD of DM and CP were determined using the index method described by Adeola (2001).

Statistical analysis

Data were analyzed as a generalized randomized block design for one-way ANOVA using the lmer function from the lme4 package in R Studio (version 3.5.2, R Core team. Vienna, Austria). Pen served as the experimental unit, dietary treatment and weight block served as fixed effects, and barn was included as a random effect. Linear and quadratic contrasts were used to test for increasing TIU. Contrast statements were used to test the main effects of treatment, day, and their interaction for fecal DM. Results were considered significant with $P < 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Results

Analysis of final diets for TIU/mg of complete feed were generally higher compared to diet formulation; however, the expected overall increase in TIU was observed for the six dietary treatments (Tables 2, 3, and 4).

In all three dietary phases and overall (d 0 to 42), increasing dietary TIU decreased (linear, $P < 0.001$) BW, ADG, ADFI, and G:F. From d 0 to 10, ADG was decreased by 36% and G:F by 22% for pigs fed 4.9 TIU/mg of complete feed compared with those fed 1.4 TIU/mg. On d 42, pigs fed 4.9 TIU/mg of complete feed were 5.6 kg lighter than those fed 1.4 TIU/mg. The decrease in ADG was partially driven by a reduction in ADFI (linear, $P < 0.001$) as TIU increased, during each phase and overall.

There was a tendency for an interaction (linear, $P = 0.094$) between treatment and day for fecal DM. On d 10, fecal DM increased (linear, $P < 0.015$; quadratic, $P = 0.088$) as TIU/mg of complete feed increased, with the majority of the increase in fecal DM observed as TIU

increased from 3.5 to 4.9 TIU/mg. However, there were no differences in fecal DM on d 42. Additionally, there was a main effect of day ($P < 0.05$) with increased fecal DM on d 42 compared with d 10.

The ATTD of DM decreased (quadratic, $P = 0.003$) as TIU of complete feed increased from 1.4 to 4.2 TIU/mg and then increased at 4.9 TIU/mg to values similar to those observed for pigs fed 2.8 TIU/mg. The ATTD of CP decreased (linear, $P < 0.001$) as the level of TIU/mg of complete feed increased in the diet. No differences were observed for d 10 plasma CCK.

Discussion

The amount of SBM in nursery diets is often limited due to ANFs that can be related to decreased nursery growth performance, which can ultimately affect profitability for producers. In this study, increasing dietary TIU concentration had a negative effect on growth performance as a result of poorer feed intake, feed efficiency, and nutrient digestibility than pigs fed the control diet. These findings are relevant given the substantial variation in TIU levels observed across US soybean meal sources in an industry survey (Gaffield et al., 2024). This wide range in SBM quality means that nursery diets may unknowingly contain high TIU levels, which is especially problematic for newly weaned pigs with limited digestive enzymatic functions that are most susceptible to ANFs. Even slight increases in dietary TIU can disproportionately decrease digestibility, nutrient utilization, and growth performance, prompting the need to determine the concentration at which performance begins to decline.

The magnitude and linear responses observed herein of responses are comparable with recent nursery pig studies. Nisley et al. (2025) and Miller et al. (2025a) both reported linear reductions in ADG, ADFI, and G:F using ground raw soybeans and soy flour to increase dietary TIU from approximately 0.5 to 6.0 and 0.61 to 3.51 TIU/mg, respectively. When combining the

results of these prior studies and the current study, each 1 TIU/mg increase in dietary TIU was associated with a 37 to 39 g/d, or 7 to 9%, reduction in overall ADG. Overall ADFI responses were also consistent, decreasing by approximately 30 to 40 g/d, or 5 to 7%, per 1 TIU/mg increase. Overall G:F declined across all three studies, with reductions ranging from approximately 3 to 6% per 1 TIU/mg increase. Importantly, dietary TIU was measured using the same assay and laboratory across all three studies, supporting direct comparison of TIU concentrations and biological responses.

In grow-finish pigs, Miller et al. (2025b) observed linear reductions in growth performance and nitrogen digestibility as dietary TIU increased from 0.99 to 9.38 TIU/mg, with final BW reduced by more than 9% and nitrogen digestibility reduced by approximately 7% at the greatest TIU concentration. The biological trajectory of these findings is remarkably similar to those in nursery pigs, in that increasing dietary TIU consistently decreases feed intake, nutrient digestibility, and growth.

In soybean processing, raw beans are dehulled, cracked, flaked, and solvent extracted to remove oil, resulting in defatted soy flakes. These soy flakes are then ground to produce soy flour or further heat processed to produce SBM. In the present study, soy flour was used as a substitute for SBM to increase dietary TIU while maintaining a constant amount of soy protein across treatments. The chemical composition of soy flour (crude protein and amino acids) analyzed similarly to soybean meal. However, soy flour has a high TIU content due to limited heat processing. This is expected because trypsin inhibitor inactivation is strongly influenced by processing conditions, including heat exposure, moisture, and treatment duration (Vagadia et al., 2017). Furthermore, soy flour was included at relatively low concentrations to achieve the

targeted TIU concentrations, minimizing potential confounding effects on diet palatability (Arndt et al., 1999) and isolating differences among diets to TIU activity.

The observed increase in d 10 fecal DM as dietary TIU increased may be partially driven by reduced feed intake, as lower ADFI can decrease digesta passage rate and increase gastrointestinal retention time (Wilfart et al., 2007), potentially allowing for greater water absorption in the hindgut and result in firmer feces. Yet the absence of fecal DM response on d 42, despite the maintained decrease in growth performance, indicates that the fecal consistency might not be a sensitive indicator of the persistent negative effects of dietary TIU as nursery pigs mature. It should be noted however, that all fecal DM values were relatively high exceeding 25% on both days 10 and 42.

Apparent total tract digestibility of DM displayed a quadratic response, with the lowest values near 4.2 TIU/mg, which may represent the point at which proteolytic inhibition most severely limits nutrient availability before partial compensatory adaptation in digestive function occurs (Goebel and Stein, 2010). Although fecal DM did not differ among treatments at d 42, ATTD of CP measured from the same fecal samples decreased linearly as dietary TIU increased, likely reflecting inhibition of trypsin and chymotrypsin activity and increased endogenous nitrogen losses. Miller et al. (2025b) and Nisley et al. (2025) confirmed similar reductions in protein digestibility when TIU concentrations exceeded 3 TIU/mg, yet the study herein shows that even values below that 3 TIU/mg have a negative effect on growth and nutrient utilization. Collectively, the present and prior data reinforce the direct link between increasing dietary TIU, enzyme inhibition, and reduced nutrient digestion.

The observed reduction in nutrient digestibility is consistent with the known physiological effects of TIU on protease activity and pancreatic function. The inhibition of

trypsin and chymotrypsin reduces enzymatic hydrolysis of dietary protein (Kunitz, 1946; Birk, 1985; Liener, 1976), thereby decreasing the normal negative feedback exerted by luminal proteases on cholecystikinin (CCK) secretion. Under these conditions, dietary TIU have been shown to stimulate CCK release and pancreatic enzyme secretion in rodents and poultry (Brand and Morgan, 1981; Temler et al., 1984; Louie et al., 1986; Clarke and Wiseman, 2005). However, despite the reduction in digestibility observed in the present study, plasma CCK concentrations were not altered by dietary TIU. This lack of response may be partially explained by the dynamic nature of circulating CCK, which is sensitive to feed intake, meal timing, and diet composition, particularly dietary fat and protein concentrations (Lhoste et al., 1995; Little et al., 2005; Karhunen et al., 2008). In the present study, reduced ADFI in pigs fed increasing dietary TIU, combined with the inability to quantify individual feed intake during the 1 h refeeding period due to group housing, may have reduced our ability to detect a treatment response on CCK. Similar inconsistencies have been previously reported in pigs, where reductions in growth performance and nutrient digestibility occur without consistent evidence of pancreatic enlargement or measurable changes in circulating CCK concentrations (Yen et al., 1974; Struthers et al., 1983). Therefore, circulating CCK may not be a sufficiently sensitive indicator of the adaptive digestive response to dietary TIU in nursery pigs. Nevertheless, compensatory increases in pancreatic enzyme secretion have been reported under these conditions (Schneeman et al., 1977; Gu et al., 2010), resulting in increased endogenous protein losses into the intestinal lumen. Because these secreted enzymes remain susceptible to inhibition by dietary TIU, a greater proportion of protein escapes digestion and absorption, ultimately reducing nitrogen utilization and apparent protein digestibility.

The data herein indicate that biologically relevant reductions in nursery pig performance can occur at relatively modest dietary TIU concentrations. In the present study, growth performance declined linearly across the evaluated range of 1.4 to 4.9 TIU/mg, indicating that relatively small increases in TIU may negatively affect nursery pig growth. Rather than identifying a single universal threshold, the present findings, together with Miller et al. (2025a) and Nisley et al. (2025), demonstrate a consistent negative response to increasing dietary TIU across multiple nursery pig studies. Likewise, Royer et al. (2022) and Miller et al. (2025b) observed that increasing dietary TIU can linearly decrease growth performance and nutrient utilization in older pigs too. Importantly, the consistency in response across studies utilizing different TIU sources, including raw soybeans, soy flour, and soybean meal-based titration models, suggests that the observed reductions in performance are primarily attributable to TIU activity rather than ingredient-specific effects.

Although the biological responses to increasing TIU are remarkably consistent across studies, reported threshold values vary considerably. Part of this variation may be attributable to differences in the analytical methodologies used to quantify inhibitor activity (Sueiro et al., 2015; Kim and Krishnan, 2023). Although TIU, TIA, and mg trypsin inhibited/g are frequently used throughout literature, these values are not always directly comparable. Trypsin inhibitor activity generally refers to the measured inhibitory activity, whereas the reported value may be expressed using different units depending on the analytical method employed. The American Association of Cereal Chemists International (AACCI) and American Oil Chemists' Society (AOCS) methods are derived from the procedure developed by Kakade et al. (1974) and quantify TIA using TIU, an arbitrary unit based on the reduction in absorbance of a chromogenic substrate. These methods have undergone several refinements over time, with Hamerstrand et al.

(1981) introducing a simplified single-dilution approach and Kim and Krishnan (2023) further reducing assay volume and analytical complexity while maintaining comparable estimates of inhibitor activity. In contrast, the ISO 14902 method determines TIA, expressed as mg trypsin inhibited/g of sample, rather than reporting TIU. Because the ISO and AACCI/AOCS methods differ in sample preparation, extraction conditions, assay chemistry, and calculation procedures, values generated by the two methodologies are not directly interchangeable. Sueiro et al. (2015) reported greater TIA values using an AACCI-based method modified by Hamerstrand et al. (1981) compared with the ISO method, while Liu (2021a) found that although ISO and AOCS derived values were highly correlated, direct numerical conversions remained difficult. Industry resources have proposed conversion factors to facilitate interpretation; however, these relationships are assay-specific and influenced by factors such as the source and activity of the trypsin reagent (Liu, 2021b). Consequently, comparisons among studies should consider both the reported TIU concentration and the analytical methodology used to generate the value. The present study utilized an AACCI/AOCS-based assay and expressed results as TIU/mg of complete feed; therefore, comparisons with studies employing similar methodologies are likely appropriate, whereas direct comparison with ISO-derived values or thresholds should be interpreted cautiously. As interest grows in establishing biologically meaningful TIU thresholds for swine diets, greater standardization of analytical methodology and reporting units will improve interpretation across studies and facilitate development of practical quality-control guidelines for soybean meal.

In summary, increasing dietary TIU decreased growth performance throughout the entire nursery period as well as decreased nutrient digestibility. Based on these results, soybean meal with greater than 5.4 TIU/mg at an inclusion similar to that used in this study would result in

TIU greater than 1.4 TIU/mg of complete feed, which will lead to a reduction in nursery growth performance and nutrient digestibility. Importantly, the 5.4 TIU/mg falls within the range of values recently reported in commercially available soybean meal sources in the US, indicating that nursery diets formulated without consideration of TIU concentration may unknowingly exceed biologically relevant levels.

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Table 1.1 Chemical analysis of soybean meal and soy flour (as-fed basis).^{1, 2}

Item	Soybean meal	Soy flour
Trypsin inhibitor units, TIU/mg	5.41	75.87
Proximate analysis, %		
Dry matter	91.92	93.04
Crude protein	48.45	50.18
Crude fat	3.00	0.62
Crude fiber	3.09	3.97
Ash	6.05	6.02
Protein dispersibility index	10.27	76.48
Total essential amino acid concentration, %		
Cys	0.71	0.74
His	1.30	1.33
Ile	2.28	2.33
Leu	3.69	3.79
Lys	3.21	3.27
Met	0.73	0.68
Thr	1.92	1.95
Trp	0.58	0.59
Val	2.38	2.40

¹ Values represent the mean of two samples analyzed in duplicate.

² Ingredient samples were submitted for complete proximate and amino acid analysis in duplicate (University of Missouri Agricultural Experiment Station Chemical Laboratory; Columbia, MO). Protein dispersibility index was analyzed in triplicate at the Kansas State University (Manhattan, KS) Swine Laboratory. Trypsin inhibitor units were analyzed in triplicate at the Agricultural Research Service, US Department of Agriculture (Columbia, MO). Analyzed values for were used in diet formulation.

Table 1.2 Phase 1 diet composition (as-fed basis).¹

Item	Trypsin inhibitor units (TIU), per mg of complete feed					
	1.4	2.1	2.8	3.5	4.2	4.9
Ingredients, %						
Corn	51.26	51.28	51.30	51.28	51.27	51.29
Soybean meal	17.57	16.55	15.53	14.55	13.56	12.54
Soy flour	0.55	1.55	2.55	3.55	4.55	5.55
Whey powder	12.50	12.50	12.50	12.50	12.50	12.50
Whey permeate, 80% lactose	7.50	7.50	7.50	7.50	7.50	7.50
Spray-dried blood plasma	2.50	2.50	2.50	2.50	2.50	2.50
Fish meal	5.00	5.00	5.00	5.00	5.00	5.00
Calcium carbonate	0.53	0.53	0.53	0.53	0.53	0.53
Monocalcium, 21% P	0.45	0.45	0.45	0.45	0.45	0.45
Salt	0.40	0.40	0.40	0.40	0.40	0.40
L-Lys-HCl	0.34	0.34	0.34	0.34	0.34	0.34
DL-Met	0.19	0.19	0.19	0.19	0.19	0.19
L-Thr	0.18	0.18	0.18	0.18	0.18	0.18
L-Trp	0.05	0.05	0.05	0.05	0.05	0.05
L-Val	0.11	0.11	0.11	0.11	0.11	0.11
L-Ile	0.03	0.03	0.03	0.03	0.03	0.03
Zinc oxide	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin premix ²	0.25	0.25	0.25	0.25	0.25	0.25
Trace mineral premix ³	0.15	0.15	0.15	0.15	0.15	0.15
Phytase ⁴	0.06	0.06	0.06	0.06	0.06	0.06
Total	100	100	100	100	100	100
Calculated analysis						
Standardized ileal digestible amino acids, %						
Lys	1.35	1.35	1.35	1.35	1.35	1.35
Ile:Lys	56	56	56	56	56	56
Leu:Lys	112	112	112	112	112	113
Met:Lys	37	37	37	36	36	36
Met and Cys:Lys	58	58	58	58	58	58

Thr:Lys	64	64	64	64	64	64
Trp:Lys	19.2	19.2	19.2	19.2	19.2	19.2
Val:Lys	70	70	70	70	70	70
His:Lys	34	34	34	34	34	34
Net energy, kcal/kg	2,518	2,518	2,518	2,518	2,518	2,518
SID Lys:net energy, g/Mcal	5.36	5.36	5.36	5.36	5.36	5.36
Crude protein, %	20.5	20.5	20.5	20.5	20.5	20.6
STTD P, %	0.57	0.57	0.57	0.57	0.57	0.57
Analyzed composition						
TIU/mg of complete feed	1.86	2.84	3.48	4.52	4.59	5.29
Crude protein, %	18.8	18.1	17.1	20.0	18.7	19.3
Dry matter, %	89.0	89.1	89.1	89.0	89.1	89.2

¹ Diets were fed from d 0 to 10.

² Provided per kg of feed: 4,134 IU of vitamin A as retinyl acetate; 1,653 IU of vitamin D₃ as cholecalciferol; 44 IU vitamin E as dl- α -tocopherol acetate; 3 mg of vitamin K as menadione; 0.03 mg of vitamin B₁₂ as cyanocobalamin; 50 mg of niacin; 26 mg of pantothenic acid as d-calcium pantothenate; 8 mg of riboflavin.

³ Provided per kg of feed: 17 mg of Cu as copper sulfate; 0.3 mg I as ethylenediamine dihydriodide; 110 mg Fe as ferrous sulfate; 33 mg Mn as manganese oxide; 0.3 mg Se as sodium selenite; 110 mg of Zn as zinc sulfate.

⁴ HiPhorius 2400 (dsm-firmenich, Plainsboro, NJ) was added at 1,500 FYT/kg feed and provided an estimated release of 0.13% STTD P.

STTD = standard total tract digestibility.

Table 1.3 Phase 2 diet composition (as-fed basis).¹

Item	Trypsin inhibitor units (TIU), per mg of complete feed					
	1.4	2.1	2.8	3.5	4.2	4.9
Ingredients, %						
Corn	58.45	58.47	58.45	58.44	58.46	58.48
Soybean meal	24.50	23.48	22.51	21.52	20.50	19.48
Soy flour	0.05	1.05	2.05	3.05	4.05	5.05
Whey powder	5.00	5.00	5.00	5.00	5.00	5.00
Whey permeate, 80% lactose	5.00	5.00	5.00	5.00	5.00	5.00
Fish meal	3.25	3.25	3.25	3.25	3.25	3.25
Calcium carbonate	0.63	0.63	0.63	0.63	0.63	0.63
Monocalcium P, 21% P	0.75	0.75	0.75	0.75	0.75	0.75
Salt	0.50	0.50	0.50	0.50	0.50	0.50
L-Lys-HCl	0.46	0.46	0.46	0.46	0.46	0.46
DL-Met	0.22	0.22	0.22	0.22	0.22	0.22
L-Thr	0.23	0.23	0.23	0.23	0.23	0.23
L-Trp	0.07	0.07	0.07	0.07	0.07	0.07
L-Val	0.15	0.15	0.15	0.15	0.15	0.15
L-Ile	0.04	0.04	0.04	0.04	0.04	0.04
Zinc oxide	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin premix ²	0.25	0.25	0.25	0.25	0.25	0.25
Trace mineral premix ³	0.15	0.15	0.15	0.15	0.15	0.15
Phytase ⁴	0.06	0.06	0.06	0.06	0.06	0.06
Total	100	100	100	100	100	100
Calculated analysis						
Standardized ileal digestible amino acids, %						
Lys	1.35	1.35	1.35	1.35	1.35	1.35
Ile:Lys	57	57	57	57	57	57
Leu:Lys	109	109	109	109	109	109
Met:Lys	39	39	39	39	39	39
Met and Cys:Lys	58	58	58	58	58	58

Thr:Lys	64	64	64	64	64	64
Trp:Lys	19.1	19.1	19.1	19.1	19.1	19.1
Val:Lys	70	70	70	70	70	70
His:Lys	34	34	34	34	34	34
Net energy, kcal/kg	2,475	2,475	2,475	2,475	2,475	2,475
SID Lys:net energy, g/Mcal	5.46	5.46	5.46	5.46	5.46	5.46
Crude protein, %	20.4	20.4	20.4	20.5	20.5	20.5
STTD P, %	0.53	0.53	0.53	0.53	0.53	0.53
Analyzed composition						
TIU/mg of complete feed	1.99	2.65	3.77	4.40	4.63	4.90
Crude protein, %	19.1	19.1	19.0	18.9	18.8	19.2
Dry matter, %	88.2	87.9	88.1	87.8	87.7	87.7

¹ Diets were fed from d 10 to 24.

² Provided per kg of feed: 4,134 IU of vitamin A as retinyl acetate; 1,653 IU of vitamin D₃ as cholecalciferol; 44 IU vitamin E as dl- α -tocopherol acetate; 3 mg of vitamin K as menadione; 0.03 mg of vitamin B₁₂ as cyanocobalamin; 50 mg of niacin; 26 mg of pantothenic acid as d-calcium pantothenate; 8 mg of riboflavin.

³ Provided per kg of feed: 17 mg of Cu as copper sulfate; 0.3 mg I as ethylenediamine dihydriodide; 110 mg Fe as ferrous sulfate; 33 mg Mn as manganese oxide; 0.3 mg Se as sodium selenite; 110 mg of Zn as zinc sulfate.

⁴ HiPhorius 2400 (dsm-firmenich, Plainsboro, NJ) was added at 1,500 FYT/kg feed and provided an estimated release of 0.13% STTD P.

STTD = standard total tract digestibility

Table 1.4 Phase 3 diet composition (as-fed basis).¹

Item	Trypsin inhibitor units (TIU), per mg of complete feed					
	1.4	2.1	2.8	3.5	4.2	4.9
Ingredients, %						
Corn	69.54	69.53	69.51	69.53	69.54	69.54
Soybean meal	25.83	24.84	23.86	22.84	21.84	20.83
Soy flour	---	1.00	2.00	3.00	4.00	5.00
Calcium carbonate	0.80	0.80	0.80	0.80	0.80	0.80
Monocalcium P, 21% P	0.95	0.95	0.95	0.95	0.95	0.95
Salt	0.60	0.60	0.60	0.60	0.60	0.60
L-Lys-HCl	0.55	0.55	0.55	0.55	0.55	0.55
DL-Met	0.23	0.23	0.23	0.23	0.23	0.23
L-Thr	0.26	0.26	0.26	0.26	0.26	0.26
L-Trp	0.08	0.08	0.08	0.08	0.08	0.08
L-Val	0.16	0.16	0.16	0.16	0.16	0.16
L-Ile	0.04	0.04	0.04	0.04	0.04	0.04
Vitamin premix ²	0.25	0.25	0.25	0.25	0.25	0.25
Trace mineral premix ³	0.15	0.15	0.15	0.15	0.15	0.15
Phytase ⁴	0.06	0.06	0.06	0.06	0.06	0.06
Titanium dioxide ⁵	0.50	0.50	0.50	0.50	0.50	0.50
Total	100	100	100	100	100	100
Calculated analysis						
Standardized ileal digestible amino acids, %						
Lys	1.30	1.30	1.30	1.30	1.30	1.30
Ile:Lys	56	56	56	56	56	56
Leu:Lys	109	109	109	109	109	110
Met:Lys	38	38	38	38	38	38
Met and Cys:Lys	58	58	58	58	58	58
Thr:Lys	64	64	64	64	64	64
Trp:Lys	19.1	19.1	19.1	19.1	19.1	19.1
Val:Lys	70	70	70	70	70	70
His:Lys	34	34	34	34	34	34

Net energy, kcal/kg	2,445	2,445	2,445	2,445	2,445	2,445
SID Lys:net energy, g/Mcal	5.32	5.32	5.32	5.32	5.32	5.32
Crude protein, %	19.3	19.3	19.3	19.3	19.4	19.4
STTD P, %	0.45	0.45	0.45	0.45	0.45	0.45
Analyzed composition						
TIU/mg of complete feed	1.74	3.07	4.01	4.70	5.21	5.19
Crude protein, %	18.2	18.4	18.0	18.2	18.7	18.3
Dry matter, %	86.9	86.9	87.0	87.2	87.0	87.0

¹ Diets were fed from d 24 to 42.

² Provided per kg of feed: 4,134 IU of vitamin A as retinyl acetate; 1,653 IU of vitamin D₃ as cholecalciferol; 44 IU vitamin E as dl- α -tocopherol acetate; 3 mg of vitamin K as menadione; 0.03 mg of vitamin B₁₂ as cyanocobalamin; 50 mg of niacin; 26 mg of pantothenic acid as d-calcium pantothenate; 8 mg of riboflavin.

³ Provided per kg of feed: 17 mg of Cu as copper sulfate; 0.3 mg I as ethylenediamine dihydriodide; 110 mg Fe as ferrous sulfate; 33 mg Mn as manganese oxide; 0.3 mg Se as sodium selenite; 110 mg of Zn as zinc sulfate.

⁴ HiPhorius 2400 (dsm-firmenich, Plainsboro, NJ) was added at 1,500 FYT/kg feed and provided an estimated release of 0.13% STTD P.

⁵ Utilized as an indigestible marker for apparent total tract digestibility calculations.

STTD = standard total tract digestibility

Table 1.5 Effects of increasing trypsin inhibitor units on nursery pig performance fecal dry matter, nutrient digestibility, and plasma cholecystokinin.¹

Item	Trypsin inhibitor units (TIU), per mg of complete feed ²						SEM	<i>P</i> =	
	1.4	2.1	2.8	3.5	4.2	4.9		Linear	Quadratic
BW, kg									
d 0	6.1	6.1	6.1	6.1	6.1	6.1	0.03	0.926	0.620
d 10	8.5	8.3	8.2	8.1	7.9	7.6	0.10	< 0.001	0.681
d 24	15.0	14.4	14.0	13.4	13.1	12.3	0.19	< 0.001	0.622
d 42	27.8	27.1	26.0	24.6	23.2	22.2	0.35	< 0.001	0.401
Phase 1 (d 0 to 10)									
ADG, g	246	220	208	200	185	157	8.1	< 0.001	0.773
ADFI, g	313	296	293	290	289	256	11.7	< 0.001	0.394
G:F, g/kg	787	738	710	692	638	613	17.6	< 0.001	0.937
Phase 2 (d 10 to 24)									
ADG, g	465	437	419	385	367	333	10.5	< 0.001	0.728
ADFI, g	636	608	595	556	536	505	13.4	< 0.001	0.712
G:F, g/kg	730	719	707	694	687	661	11.4	< 0.001	0.602
Phase 3 (d 24 to 42)									
ADG, g	708	693	663	621	564	553	11.5	< 0.001	0.383
ADFI, g	1,133	1,108	1,095	1,041	967	943	17.5	< 0.001	0.144
G:F, g/kg	625	626	607	598	583	587	7.4	< 0.001	0.600
Overall (d 0 to 42)									
ADG, g	517	491	474	442	408	385	0.018	< 0.001	0.488
ADFI, g	772	741	737	700	662	633	0.025	< 0.001	0.236

G:F, g/kg	669	662	644	632	617	609	0.015	< 0.001	0.870
Fecal dry matter, % ³									
d 10	25.87	25.42	25.44	25.83	26.80	27.59	0.637	0.015	0.088
d 42	27.46	26.87	26.15	27.65	26.36	27.54	0.637	0.943	0.261
Apparent total tract digestibility, %									
Dry matter ⁴	85.65	84.27	84.69	83.26	80.74	84.88	0.647	0.001	0.003
Crude protein ⁴	79.87	77.19	77.55	75.22	75.22	75.55	0.952	< 0.001	0.105
Plasma cholecystokinin, pg/mL									
d 10	257	234	235	262	310	246	30.8	0.273	0.948

¹A total of 360 barrows (Line 200 × 400, DNA, Columbus NE, initially 6.1 ± 0.03 kg) were used with five pigs per pen and 12 replicates per treatment.

² Soy flour and soybean meal were blended to create the TIU/mg of complete feed for dietary treatments.

³ Feces from three pigs per pen were weighed and dried to measure fecal dry matter. Linear treatment × day interaction, $P = 0.094$; Treatment, $P = 0.120$; Day, $P = 0.023$.

⁴ Both ground feed and fecal samples were dried at 135°C for 2 h to determine percentage dry matter of the samples used for titanium determination. Titanium dioxide concentration in both dried feed and fecal samples was analyzed in duplicate.

Chapter 2 - An industry survey of the composition and variability of soybean meal across US soybean processing plants

Abstract

Soybean meal is the primary protein source used in US monogastric diets; however, updated, large scale surveys describing its current composition and variability across US soybean processing plants are limited. This survey included 27 solvent extracted plants, representing approximately half of the US solvent extraction soy processing plants, and 2 extruded-expelled plants, ensuring a representative range in crush capacity and geographical location. Samples were collected at two timepoints within the same crop year (from October 2024 to January 2025 and from June to August 2025). At each timepoint, 5 samples were collected over the course of one month to provide a thorough view of the soybean meal composition at that facility. Therefore, 271 soybean meal samples in total were received for analysis. Samples were analyzed in duplicate for proximate analysis, neutral detergent fiber, oligosaccharides, fatty acid profile, amino acid profile, protein dispersibility index (PDI), potassium hydroxide (KOH) protein solubility, trypsin inhibitor units (TIU), urease activity, mineral analysis, bulk density, and flowability. Overall, soybean meal composition was noticeably consistent between seasonal sampling timepoints; however, processing method largely affected the composition of the soybean meal. As expected, solvent extracted soybean meal had higher crude protein (47 vs 42%), lower ether extract (1.9 vs 9.5%), and lower trypsin inhibitor units (4.5 vs 14.7 TIU/mg) compared to extruded-expelled soybean meal. Furthermore, measurable variation was observed, both between and within processing plants, particularly in ether extract, Ca, TIU, KOH, PDI, and particle size. For solvent extraction plants, ether extract ranged from 0.73 to 5.22%, Ca ranged from 0.18 to 0.84%, TIU ranged from 0.61 to 9.58 TIU/mg, KOH ranged from 74.82 to 93.65%,

PDI ranged from 8.60 to 48.37%, and particle size ranged from 546 to 1,508 μm . However, other analyses, notably crude protein, amino acids, and fatty acids showed less variability, both between and within processing plants. For samples collected from solvent extraction plants, the average crude protein was $46.9 \pm 0.97\%$ with lysine averaging $3.07 \pm 0.12\%$. These findings provide an updated industry benchmark describing the current composition and variability of US soybean meal to support precision diet formulation, quality assessment, and informed collaboration between soybean processors and animal nutritionists.

Key Words: analytical composition, extruded-expelled, solvent extraction, soybean meal quality, soybean processing, trypsin inhibitor

Introduction

Soybean meal (SBM) is the predominant plant-based protein source used in animal diets. Among plant protein ingredients, SBM is commonly considered the reference source against which alternative protein ingredients are evaluated because of its favorable amino acid profile, high nutrient digestibility, widespread availability, and relatively low cost (Nahashon and Kilonzo-Nthenge, 2011; NRC, 2012; Ibáñez et al., 2020; Ruiz et al., 2020). In the soybean marketing year 2024/2025, approximately 36 million metric tons of SBM were used in animal feeds, with 74% of that in poultry and swine diets (United Soybean Board, 2026). Therefore, understanding SBM composition, processing quality, and variability across soybean processing plants is a critical area of focus in monogastric nutrition.

Previous evaluations of commercial SBM sources have reported variation in nutrient composition and quality characteristics among processing facilities, including differences in

amino acid concentrations, oligosaccharide content, residual oil concentration, and other compositional attributes (Cromwell et al., 1999; Grieshop et al., 2003; Karr-Lilienthal et al., 2005; Gaffield et al., 2024b). These differences may arise from variation in soybean genetics and environmental growing conditions, as well as processing practices such as dehulling, oil extraction efficiency, thermal processing conditions, and plant-specific manufacturing procedures (Wolf et al., 1982; Wilcox and Shibles, 2001; Brumm and Hurburgh, 2006; Karr-Lilienthal et al., 2006). Furthermore, incorporation of soybean processing byproducts and differences in extraction methodology among facilities may contribute to variation in the final nutrient composition and physical characteristics of SBM (Witte, 1995; Gaffield et al., 2024b). Consequently, variation in SBM composition and processing characteristics may influence ingredient quality, nutrient value, and formulation accuracy in animal diets.

Historical surveys have provided valuable benchmarks for SBM composition; however, continued changes in soybean production, processing technologies, and manufacturing practices may contribute to differences in SBM composition and quality compared with historical reference values commonly used in feed formulation systems. Furthermore, recent expansion of US soybean crushing capacity has increased the number and diversity of processing facilities contributing to the domestic SBM supply (Economic Research Service, USDA, 2026; United Soybean Board, 2026). Consequently, updated characterization of SBM composition, processing quality, and variability are needed to ensure industry values accurately reflect commercially available products. However, contemporary large-scale surveys comprehensively characterizing the nutrient composition, processing quality indicators, physical characteristics, and variability of SBM across US soybean processing facilities remain limited.

Therefore, the objective of this industry survey was to characterize the nutrient composition, processing quality indicators, physical characteristics, and variability of SBM collected from soybean processing facilities across the US. Understanding between and within plant variability in nutrient composition and physical characteristics of SBM is increasingly important for ingredient evaluation, feed manufacturing, quality assurance, and accurate diet formulation across animal feeding systems. Additionally, differences between solvent extracted and extruded-expelled SBM sources, plant crush capacity, and consistency across seasonal sampling timepoints were evaluated to provide an updated industry overview of SBM composition and variability.

Materials and methods

A total of 271 SBM samples from 29 soybean processing plants across 16 different companies were used in this industry survey. Twenty-seven of the soybean processing plants sampled were solvent extracted, while two plants were mechanically extruded-expelled. The individual solvent extracted plants surveyed constitute approximately 40% of total US SBM production, while the 16 companies represent 90% of the total US SBM production. For evaluation of plant size, solvent extracted processing facilities were categorized by daily soybean crush capacity in metric tons (MT) per day as small (< 2,500 MT/day; n = 6), medium (2,500 to 4,500 MT/day; n = 9), or large (> 4,500 MT/day; n = 9). Soybean meal samples were collected at two different timepoints within the same crop year: October 2024 to January 2025 and from June to August 2025. At each timepoint, 5 samples (2.25 kg each) were collected over the course of one month to provide a thorough view of the SBM composition at that facility. Two plants within the survey only provided samples represented at timepoint 1 and one plant only provided

samples represented at timepoint 2. Three plants had either three or four samples represented at timepoint 2, as samples were not received by the deadlines set for this study.

Plants received pre-labeled sampling bags (Whirl-Pak, Nasco, Ft. Atkinson, WI) at each timepoint of collection. Upon receipt of the sampling packages, an employee from the soybean processing plant was asked to collect samples across five separate days throughout a month for both timepoints. Information regarding the date of collection, employee name and contact information, and whether a soybean oil refinery was located adjacent to the plant was recorded. Once collected, the samples were shipped to Kansas State University to be prepared for analysis. All samples were stored at room temperature until analyses were performed. Each sample was homogenized through a riffle splitter and analyzed for bulk density, angle of repose, and particle size. Prior to shipment for analysis, samples were ground to approximately 220 μm and divided into two subsamples to be analyzed individually. Values reported represent an individual analysis for each subsample (mean $\pm$ standard deviation; range).

Chemical analysis

Soybean meal samples were analyzed for bulk density (Method C29-07; ASTM, 2007), angle of repose (Groesbeck et al., 2006), urease activity (Method Ba 9-58; AOCS, 2017), and particle size (Stark and Kalivoda, 2016) at the Kansas State University Swine Laboratory (Manhattan, KS). Particle size was determined using a representative 100 g sample with 0.5 g added flow agent and a 13-sieve US Standard sieve stack fitted with rubber balls and bristle sieve agitators. The sieve stack was then shaken for 10 minutes using Tyler Ro-tap (Model RX-29-E; W.S. Tyler, Mentor, OH). Ground soybean meal samples were analyzed in duplicate for each analysis at the University of Missouri Agricultural Experiment Station Chemical Laboratory (Columbia, MO). Proximate composition was determined including dry matter (Method 934.01;

AOAC, 2006), crude protein (Method 990.03; AOAC, 2006), ether extract (Method 920.39; AOAC, 1998), crude fiber (Method 978.10, AOAC, 2006), and ash (Method 942.05; AOAC, 2005), as well as neutral detergent fiber (ANKOM Technology, 2017). Complete amino acid profile excluding tryptophan (Method 982.30 E (a, b, c); AOAC, 2006), tryptophan by alkaline hydrolysis (Method 988.15, AOAC, 2006), fatty acid profile using capillary gas-liquid chromatography (Method 996.06; AOAC, 2001), mineral analysis by inductively coupled plasma optical emission spectroscopy (Method 985.01 A, B, and D; AOAC, 2005), SBM oligosaccharides including sucrose, raffinose, stachyose, and verbascose (Janauer and Englmaier, 1978), protein dispersibility index (Method 46-24; AACC, 2006), and potassium hydroxide (KOH) protein solubility (Parsons et al., 1991) were also analyzed in duplicate. Each ground SBM sample was submitted for analysis of trypsin inhibitor units in triplicate at Agricultural Research Service, US Department of Agriculture (Columbia, MO) utilizing procedures outlined by Kim and Krishnan (2023). All results are reported on an as-is basis.

Statistical analysis

Descriptive statistics were used to show the simple means and standard deviation of each analytical criteria within SBM type and timepoint of sample collection. Additionally, the range was included, which represented the minimum and maximum values for each analytical criteria within the SBM type and timepoint of sample collection. The range was based on an individual analysis and does not represent the values of a sample analyzed in duplicate.

Results and discussion

This survey provides an updated evaluation of SBM composition, processing quality, and physical characteristics across soybean processing facilities in the US. The facilities included in this survey represented approximately 40% of total US SBM production and encompassed a

wide range of geographical locations, crush capacities, and extraction methodologies representative of the industry.

Previous research has demonstrated regional differences in soybean protein and oil concentrations across major US soybean producing regions (Brumm and Hurburgh, 2006). However, while soybeans may vary in chemical composition across geographical regions, Sotak-Peper et al. (2017) reported only minor differences in amino acid composition and digestibility among soybean meal sources collected from crushing facilities throughout the US, suggesting that the nutritional value of commercially produced soybean meal remains relatively consistent across regions. Furthermore, many commercial processing facilities source soybeans from multiple production regions, transporting soybeans across state lines when local supplies are insufficient, and subsequently storing the soybeans in bins that do not allow for segregation by origin. Therefore, soybean meal crush plant location was not evaluated in this survey as geographic location of the soybeans could not be separated from location of the crush plant.

Overall, SBM composition was relatively consistent between sampling timepoints within the same crop year, suggesting limited seasonal influence on most nutritional characteristics. However, notable differences between solvent extracted and extruded-expelled soybean meal were observed, particularly for residual oil concentration, fiber content, processing quality indicators, and physical characteristics; which were expected due to the fundamental differences in these oil extraction systems. Despite these differences between processing techniques, proximate composition and amino acid profiles for both solvent extracted and extruded-expelled SBM were generally consistent with expected reference values (NRC, 2012).

Proximate composition

Differences in proximate composition were observed between SBM derived from solvent extracted and extruded-expelled processing systems (Table 1). Solvent extracted SBM contained lower dry matter, ether extract (Figure 1), crude fiber, and neutral detergent fiber (Figure 2), but higher crude protein concentration compared with extruded-expelled SBM. Crude protein averaged $46.9 \pm 0.97\%$ for solvent extracted and $42.4 \pm 2.47\%$ for extruded-expelled SBM. Within solvent extracted plants, crude protein concentration was generally consistent across crush capacity categories, averaging 47.3 ± 1.14 , 46.8 ± 0.95 , and $46.7 \pm 0.76\%$ for small, medium, and large crush capacity facilities, respectively (Figure 3). Ether extract averaged $1.90 \pm 0.62\%$ in solvent extracted SBM compared with $9.52 \pm 0.91\%$ in extruded-expelled soybean meal. These differences primarily reflect the reduced efficiency of mechanical oil extraction compared with solvent extraction, resulting in greater residual oil remaining in the meal fraction (Witte, 1995; Woerfel, 1995a). The increased residual oil concentration dilutes protein concentration, contributing to the lower crude protein values observed in extruded-expelled facilities. Variability between processing plants was evident, with solvent extracted SBM ranging from 0.73 to 5.22% ether extract. This variability likely reflects differences in efficiency of oil extraction and the addition of soybean byproducts such as soybean gums and soapstocks (Woerfel, 1995b; Gaffield et al., 2024b). Within crush capacity categories of solvent extracted plants (Figure 3), small plants had a greater ether extract ($2.33 \pm 0.77\%$) than medium ($1.81 \pm 0.41\%$) and large plants ($1.76 \pm 0.59\%$). This may be a result of medium and large plants having newer equipment and likely more efficient oil extraction than small plants.

Neutral detergent fiber concentrations averaged $7.28 \pm 1.35\%$ across sampling timepoints for solvent extracted SBM and $9.70 \pm 1.17\%$ for extruded-expelled SBM. Although average

NDF concentrations were relatively consistent, a wide range of 4.41 to 13.19% was observed within individual solvent extracted samples. Fiber concentration in SBM is largely influenced by the extent of dehulling prior to oil extraction, as soybean hulls contain substantially greater concentrations of structural carbohydrates than the cotyledon fraction (Stein et al., 2008). The efficiency of dehulling and subsequent hull aspiration may also be influenced by the moisture content of incoming soybeans. Although soybean hulls are not commonly added back to SBM in modern production, the quantity of residual hulls remaining after cleaning, dehulling, and aspiration may vary among processing facilities (Gaffield et al., 2024a). Consequently, differences in dehulling and hull aspiration efficiency of soybeans between processing facilities likely contributed to the observed variability in NDF concentration, with greater residual hull content resulting in increased fiber concentrations. Collectively, these findings indicate that variation in dehulling and oil extraction practices remains an important source of variability in the proximate composition of commercially produced SBM.

Amino acid profile

Differences in amino acid composition generally reflected differences in crude protein concentration between processing methods (Table 2). Amino acid concentrations were relatively consistent across sampling timepoints and exhibited comparatively little variability both within and between processing plants. For solvent extracted SBM, lysine averaged $3.07 \pm 0.12\%$, methionine averaged $0.65 \pm 0.03\%$, and threonine averaged $1.86 \pm 0.10\%$ across timepoints. Similar trends were observed for the remaining essential and non-essential amino acids. Amino acid profiles were generally consistent within processing methods and sampling timepoints. For crush capacity, there were no differences in amino acids for solvent extracted SBM. For example, lysine averaged 3.07 ± 0.09 , 3.06 ± 0.11 , and $3.09 \pm 0.13\%$ for small, medium, and

large crush capacity plants, respectively (data not shown). Although amino acid concentrations may vary with crude protein concentration and residual oil content, previous studies have demonstrated that differences in processing method and extent of heating can influence amino acid digestibility and nutritional value of SBM (Karr-Lilienthal et al., 2006; Opapeju et al., 2006; Powell et al., 2011). Lysine, methionine, threonine, and other nutritionally important amino acids for swine and poultry remained within expected ranges and aligned closely with historical reference values reported by NRC (2012) and previous surveys of SBM composition (Cromwell et al., 1999; Grieshop et al., 2003; Karr-Lilienthal et al., 2005). The relatively consistent amino acid composition observed across processing methods, sampling timepoints, and crush capacity categories reinforces the value of SBM as a reliable protein source for monogastric diet formulation and supports its continued use as the reference ingredient against which alternative protein sources are commonly evaluated.

Fatty acid profile

Fatty acid profiles were generally similar between solvent extracted and extruded-expelled SBM across both sampling timepoints (Table 3). Polyunsaturated fatty acids comprised approximately 60% of total lipids, regardless of processing method. This profile closely reflects that of soybean oil, indicating minimal alteration of lipid composition during processing. Although minor differences in saturated and monounsaturated fatty acid proportions were observed, these were small relative to differences in ether extract concentration, reinforcing that processing method primarily influenced residual oil concentration rather than fatty acid composition. For crush capacity of solvent extracted plants, fatty acid profiles did not differ (data not shown). These findings indicate that oil extraction methods primarily influence the quantity of residual oil retained within SBM rather than its fatty acid composition, suggesting that

variation in ether extract concentration may have greater implications for dietary energy contribution than fatty acid profile.

Mineral composition

Mineral concentrations were generally consistent for solvent extracted and extruded-expelled SBM (Table 4). Levels of macro and microminerals (Ca, K, Mg, Na, P, Cu, Fe, Mn, and Zn) generally agreed with those reported in NRC (2012). However, Ca exhibited considerable variability in solvent extracted soybean meal, ranging from 0.18 to 0.84%, whereas Ca concentrations were lower and less variable in extruded-expelled soybean meal, ranging from 0.21 to 0.33% (Figure 4). Evaluation by processing plant indicated that Ca variability occurred both within and between solvent extracted processing plants, as some facilities exhibited relatively consistent within plant Ca concentrations and others showed substantial within plant variation. Differences in average Ca concentration between processing plants may be partially explained by the inclusion of limestone as a flow agent in SBM, with allowable inclusion rates up to 0.5% (NOPA, 2025), which is consistent with previous reports attributing elevated Ca concentrations in SBM to limestone addition during handling and storage (Batal et al., 2010). Soybean meal on average contains $0.33 \pm 0.10\%$ Ca (NRC, 2012), and the addition of 0.5% limestone containing approximately 38% Ca would contribute an additional 0.19% Ca, increasing the expected Ca concentration of SBM to about 0.52%. Therefore, the addition of limestone largely explains this between plant variation. When categorized by crush capacity, Ca concentration averaged 0.36 ± 0.14 , 0.38 ± 0.11 , and $0.42 \pm 0.13\%$ for small, medium, and large solvent extracted facilities, respectively (Figure 3). It is hypothesized that the large soy processing plants are more efficient at oil extraction; therefore, they can increase add backs such as limestone as a flow agent while still achieving their crude protein targets. Collectively, these

results suggest that Ca concentration may be more influenced by manufacturing practices than most other mineral constituents and highlight the importance of routine analysis when estimating the nutrient contribution of SBM for use in diet formulation.

Carbohydrate composition

Sucrose, stachyose, raffinose, and verbascose concentrations were generally consistent across processing methods and sampling timepoints, indicating limited seasonal influence on SBM carbohydrate composition (Table 5). Carbohydrate concentrations observed in the present survey were comparable to those reported by Karr-Lilienthal et al. (2005). The relatively consistent concentrations of sucrose, stachyose, raffinose, and verbascose observed are also consistent with previous reports indicating that soybean carbohydrate composition is relatively stable across production regions and growing conditions (Grieshop et al., 2003). Because carbohydrates are primarily associated with soybean seeds and are not affected by oil extraction methodology, relatively little variation among processing methods was expected. This was consistent with the lack of differences in carbohydrate composition among crush capacity categories within solvent extracted facilities (data not shown). The consistency in the analyzed carbohydrate concentration observed in the present survey suggests that commercially available SBM remains a relatively stable source of fermentable carbohydrates for monogastric diets.

Processing quality indicators

Processing quality indicators differed substantially between SBM processing methods (Table 6). Notably, extruded-expelled SBM had considerably greater TIU concentrations, ranging from 7.84 to 20.68 TIU/mg, compared with solvent extracted soybean meal, which ranged from 0.61 to 9.58 TIU/mg (Figure 5). This observation is consistent with differences in thermal processing associated with the two oil extraction systems. Solvent extraction includes a

relatively standardized desolventizing and toasting process designed to reduce antinutritional factors, whereas heat exposure during extrusion and mechanical oil extraction may vary considerably among facilities. Consequently, differences in thermal processing intensity likely contributed to the wide range of trypsin inhibitor concentrations between and within SBM plants. Elevated trypsin inhibitor concentrations are of practical importance because inadequate heat treatment can reduce protein utilization and animal performance through inhibition of trypsin and chymotrypsin activity (Rackis, 1974; Liener, 1981; Vagadia et al., 2017). Previous surveys have reported similar variation in trypsin inhibitor concentrations among solvent extracted SBM sources, indicating that thermal processing remains an important consideration for SBM evaluation for the animal nutrition industry (Gaffield et al., 2024b). Within the present survey, one processing plant produced samples ranging from 1.98 to 9.58 TIU/mg during timepoint 2, demonstrating that substantial variation in thermal processing can occur even within a single facility. When categorized by crush capacity, mean TIU concentrations were relatively similar among solvent extracted facilities, averaging 5.19 ± 2.17 , 4.07 ± 1.02 , and 4.92 ± 1.52 TIU/mg for small, medium, and large facilities, respectively (Figure 3). However, small facilities had the greatest variability, ranging from 0.61 to 9.58 TIU/mg, compared with ranges of 2.26 to 6.24 TIU/mg and 2.53 to 7.70 TIU/mg for medium and large facilities, respectively. This greater variability may reflect differences in processing equipment or thermal processing control, as smaller facilities may be more representative of older plants compared with newer facilities that typically have greater crush capacities. Additional indicators of proper thermal processing were generally similar between solvent extracted and extruded-expelled processing methods. Protein solubility in potassium hydroxide (KOH) averaged $86.5 \pm 3.73\%$ and $86.4 \pm 2.88\%$ for solvent extracted and extruded-expelled SBM, respectively, while protein dispersibility index (PDI)

averaged $21.1 \pm 7.17\%$ and $16.9 \pm 5.54\%$. Urease activity (UA) averaged 0.26 ± 0.40 and $0.94 \pm 0.69 \Delta \text{pH}$ units for solvent extracted and extruded-expelled SBM, respectively, and ranged from 0.03 to 2.20 and 0.12 to 1.70 pH units. Although mean KOH and PDI values generally fell within ranges considered indicative of adequately processed SBM, substantial variability was observed between and within plant, with KOH ranging from 74.82 to 93.65% and PDI ranging from 8.60 to 48.37%. When categorized by crush capacity, PDI and KOH remained relatively consistent across crush capacity categories (data not shown). Previous industry guidance has suggested ideal ranges of approximately 73 to 86% for KOH protein solubility and 15 to 30% for PDI in adequately processed SBM (USSEC, 2021). Values below these ranges generally indicate excessive heat exposure and reduced protein solubility, which may be accompanied by reduced amino acid availability and animal performance, whereas values above these ranges may suggest insufficient heat treatment (Parsons et al., 1992; USSEC, 2021). Several SBM sources evaluated in the present survey fell outside these recommended ranges, indicating a need for greater focus on soybean meal quality characteristics in the future when considering utilization in animal diets.

Importantly, no single quality indicator alone completely characterizes heat treatment. Protein solubility in KOH is most useful for identifying overprocessed SBM, as protein solubility generally decreases with increasing thermal exposure and heat-induced changes in protein structure (Araba and Dale, 1990; Parsons et al., 1991). However, KOH solubility is less useful for distinguishing adequately processed SBM from underprocessed SBM because protein solubility may remain high or respond inconsistently during early stages of heat treatment. In contrast, urease activity is primarily useful for identifying underprocessed SBM but becomes less sensitive once adequate heat treatment has been achieved; therefore, it has limited ability to distinguish adequately processed from overcooked SBM (Caskey and Knapp, 1944; Araba and

Dale, 1990; Batal et al., 2000). Protein dispersibility index has been reported to respond more consistently across increasing heat treatment and may be a more sensitive indicator of minimum adequate processing than either urease activity or KOH solubility (Batal et al., 2000).

Collectively, these measures, along with trypsin inhibitor concentration, provide complementary information regarding the effects of thermal processing on SBM quality. Therefore, evaluating multiple processing indicators simultaneously may provide a more comprehensive assessment of processing adequacy than reliance on any single analytical measure.

Physical characteristics

Physical characteristics also differed substantially between solvent extracted and extruded-expelled processing methods and may have important implications for feed manufacturing (Table 7). Extruded-expelled SBM exhibited greater angle of repose values and lower bulk density compared with solvent extracted SBM, indicating poor flowability. Extruded-expelled SBM also tended to have a smaller particle size than solvent extracted soybean meal, although substantial variability was observed among solvent extracted processing plants (Figure 6). Particle size ranged from 546 to 1,508 μm among solvent extracted sources and from 335 to 927 μm among extruded-expelled sources. These physical differences are likely attributable to the greater residual oil content and mechanical processing associated with extruded-expelled SBM (Lawrence et al., 2003). Residual oil may increase particle adhesion and reduce ingredient flow characteristics, thereby affecting handling, storage, transportation, and feed manufacturing operations. Additionally, the considerable variation in particle size observed among solvent extracted SBM sources may further influence ingredient flowability and mixing characteristics within feed manufacturing systems. However, physical characteristics did not differ among crush capacity categories within solvent extracted facilities (data not shown). These findings indicate

that extraction methodology influences not only nutrient composition but also the physical properties of SBM, which may affect ingredient handling and manufacturing performance across feed production systems.

Conclusions

Collectively, the results of this survey demonstrate that contemporary US SBM remains a highly consistent source of protein and amino acids for animal feeding programs, with relevance across livestock, poultry, aquaculture, and companion animal feed applications. Overall, seasonal variation in SBM composition was minimal. However, substantial differences were observed for processing-related characteristics including residual oil concentration, trypsin inhibitor concentration, protein quality indicators, Ca concentration, and physical characteristics such as particle size. These findings indicate notable differences in processing procedures and potential incoming soybean quality among facilities suggesting that nutrient composition alone may not fully characterize SBM quality. Routine evaluation of processing quality indicators and physical characteristics, in addition to traditional nutrient analyses, may improve ingredient assessment and support more informed decision-making by nutritionists and soybean processors. Overall, this survey provides an updated assessment of the composition and quality characteristics of SBM currently available throughout the US feed industry.

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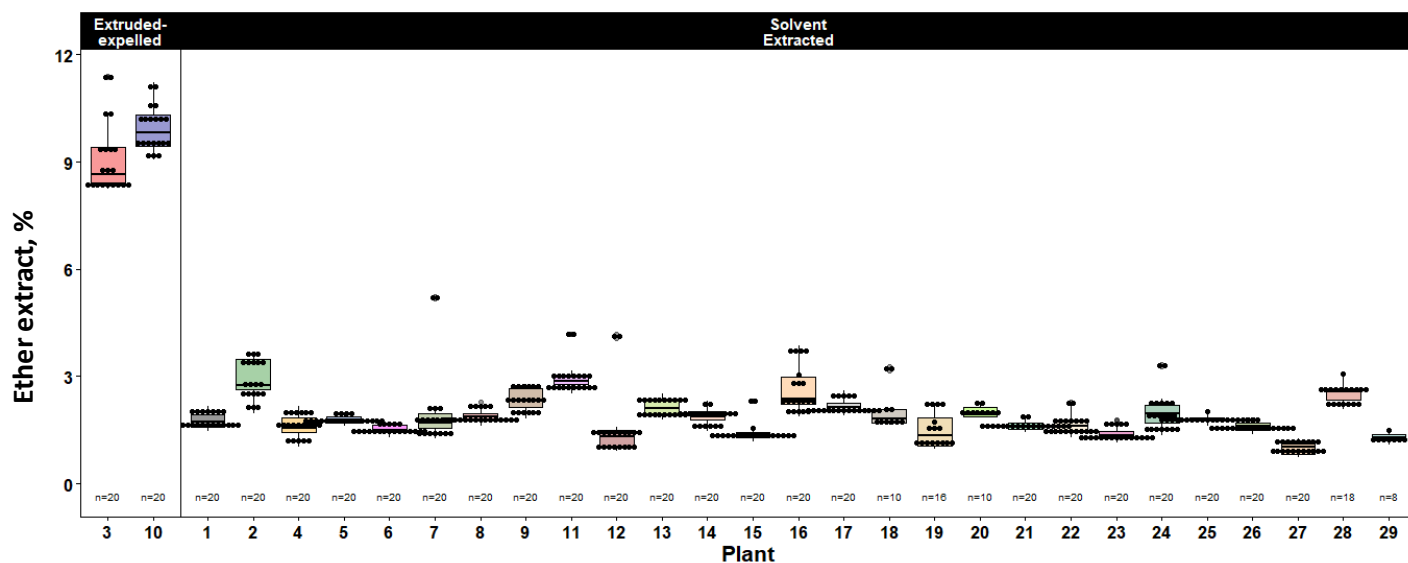


Figure 2.1 Ether extract of soybean meal samples by soybean processing plant and soybean oil extraction type (as-is basis). Individual boxplots are presented for each soybean processing plant where each data point represents a sample analyzed in singlet. The line within the boxplot represents the median, the lower and upper bounds represent the 25th and 75th percentiles, respectively, the lower and upper whiskers represent the minimum and maximum data points within 1.5 times the interquartile range, and individual data points outside the whiskers represent points exceeding 1.5 times the interquartile ranges.

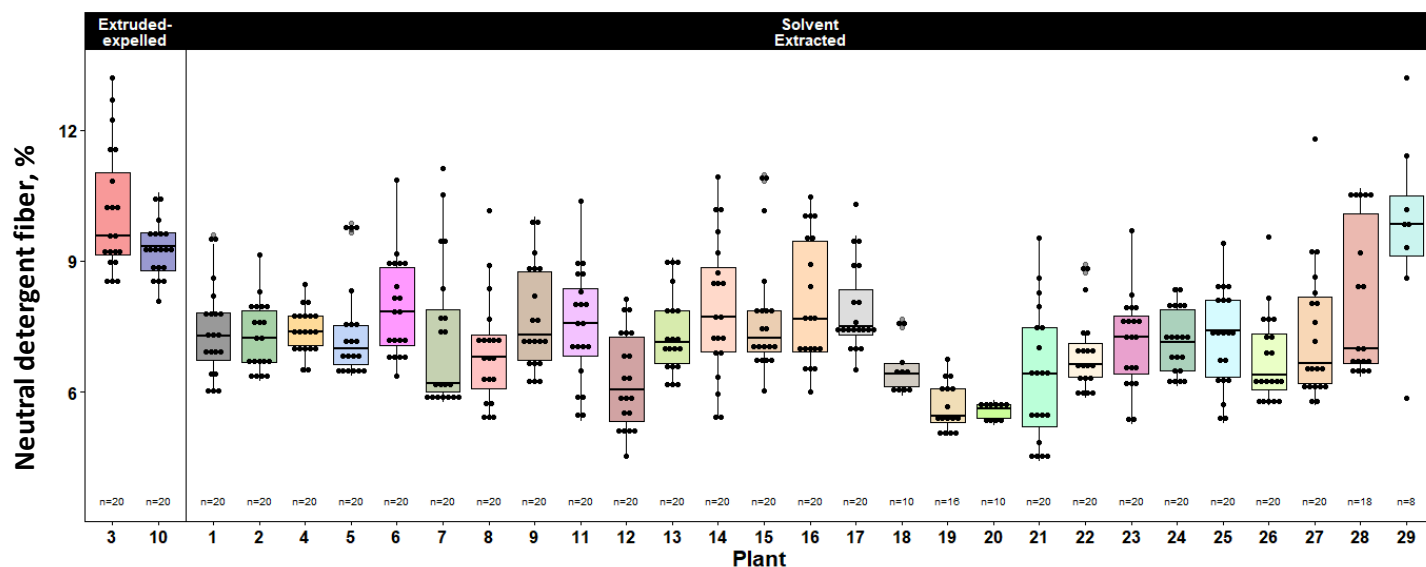


Figure 2.2 Neutral detergent fiber of soybean meal samples by soybean processing plant and soybean oil extraction type (as-is basis). Individual boxplots are presented for each soybean processing plant where each data point represents a sample analyzed in singlet. The line within the boxplot represents the median, the lower and upper bounds represent the 25th and 75th percentiles, respectively, the lower and upper whiskers represent the minimum and maximum data points within 1.5 times the interquartile range, and individual data points outside the whiskers represent points exceeding 1.5 times the interquartile ranges.

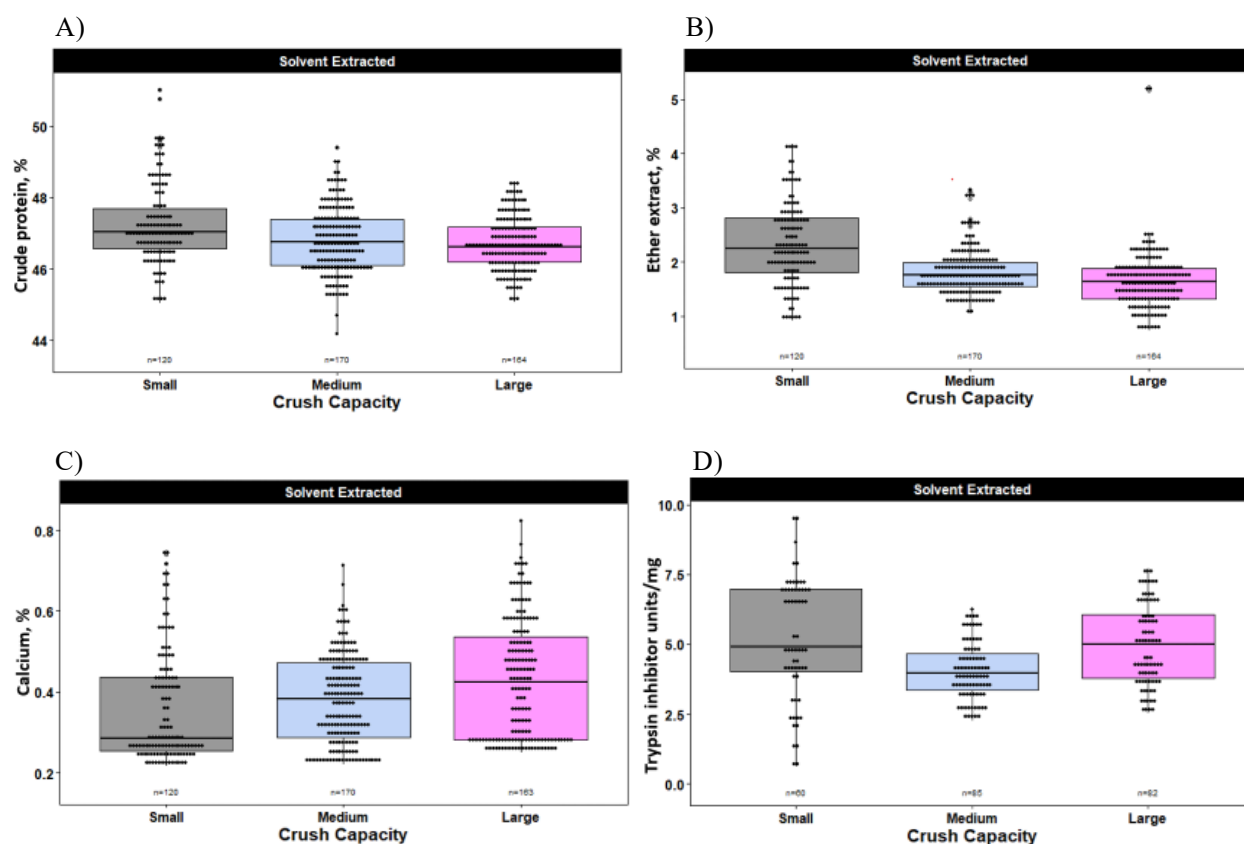


Figure 2.3 Soybean meal composition and processing quality indicators categorized by solvent extracted facility crush capacity. A) crude protein, B) ether extract, C) calcium, and D) trypsin inhibitor concentration. Soybean processing facilities were categorized by daily soybean crush capacity in metric tons (MT) per day; small (< 2,500 MT/day), medium (2,500 to 4,500 MT/day), and large (> 4,500 MT/day). Each data point represents a sample analyzed in singlet. The line within the boxplot represents the median, the lower and upper bounds represent the 25th and 75th percentiles, respectively, the lower and upper whiskers represent the minimum and maximum data points within 1.5 times the interquartile range, and individual data points outside the whiskers represent points exceeding 1.5 times the interquartile ranges.

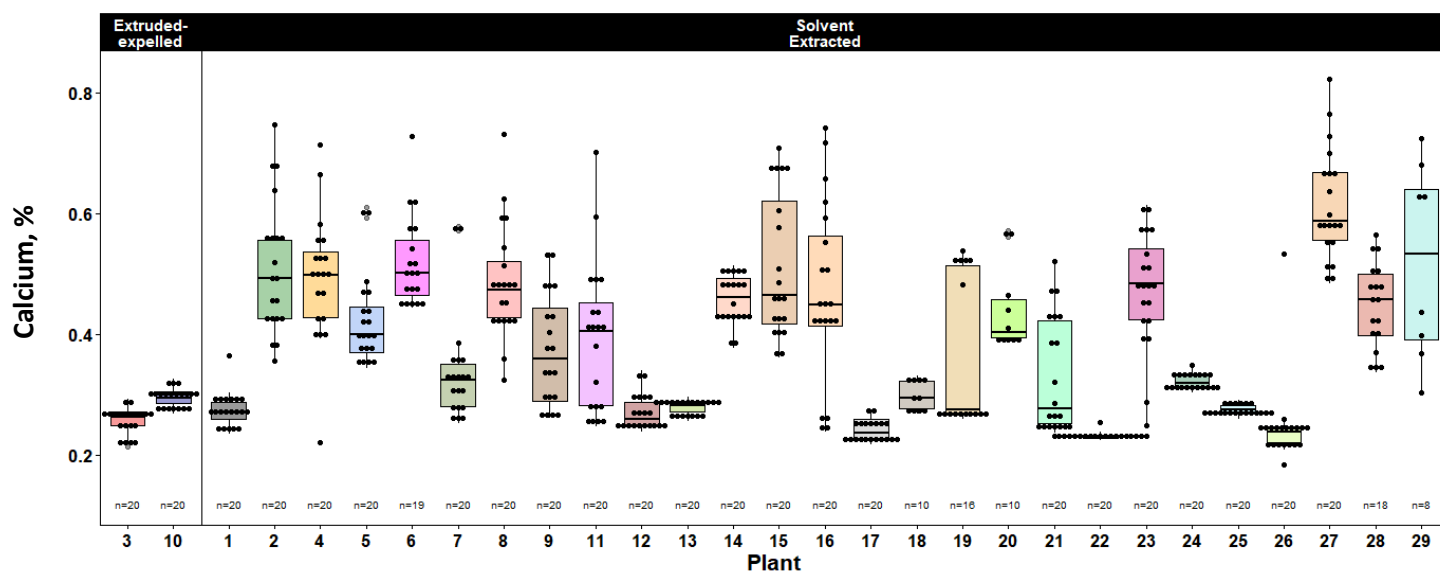


Figure 2.4 Calcium of soybean meal samples by soybean processing plant and soybean oil extraction type (as-is basis). Individual boxplots are presented for each soybean processing plant where each data point represents a sample analyzed in singlet. The line within the boxplot represents the median, the lower and upper bounds represent the 25th and 75th percentiles, respectively, the lower and upper whiskers represent the minimum and maximum data points within 1.5 times the interquartile range, and individual data points outside the whiskers represent points exceeding 1.5 times the interquartile ranges.

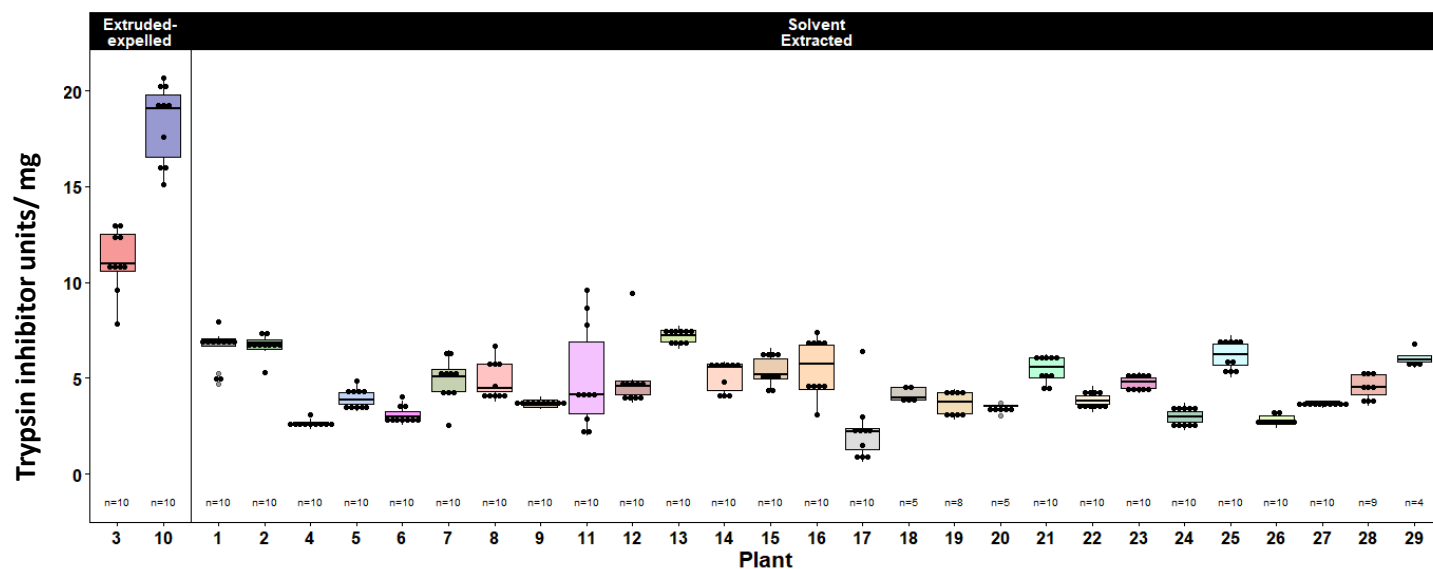


Figure 2.5 Trypsin inhibitor units of soybean meal samples by soybean processing plant and soybean oil extraction type (as-is basis). Individual boxplots are presented for each soybean processing plant where each data point represents a sample analyzed in singlet. The line within the boxplot represents the median, the lower and upper bounds represent the 25th and 75th percentiles, respectively, the lower and upper whiskers represent the minimum and maximum data points within 1.5 times the interquartile range, and individual data points outside the whiskers represent points exceeding 1.5 times the interquartile ranges.

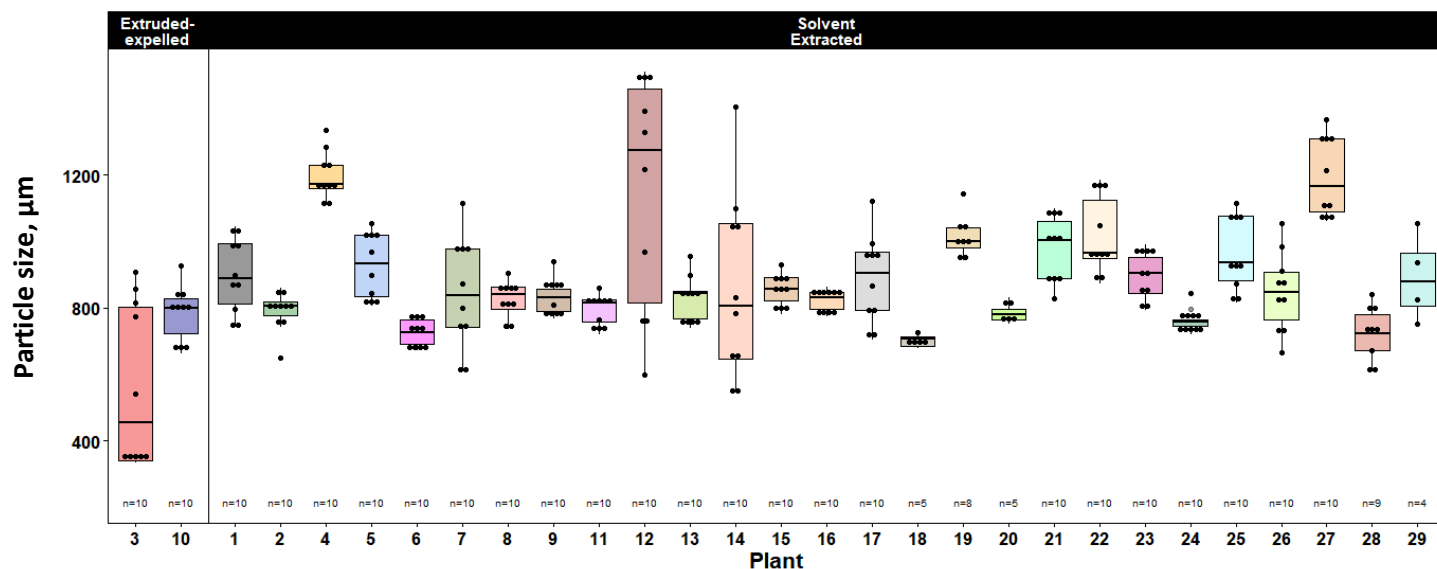


Figure 2.6 Particle size of soybean meal samples by soybean processing plant and soybean oil extraction type (as-is basis). Individual boxplots are presented for each soybean processing plant where each data point represents a sample analyzed in singlet. The line within the boxplot represents the median, the lower and upper bounds represent the 25th and 75th percentiles, respectively, the lower and upper whiskers represent the minimum and maximum data points within 1.5 times the interquartile range, and individual data points outside the whiskers represent points exceeding 1.5 times the interquartile ranges.

Table 2.1 Soybean meal proximate composition by type and sampling timepoint (as-is basis).^{1,2}

Item, %	Solvent extracted				Extruded-expelled			
	Timepoint 1		Timepoint 2		Timepoint 1		Timepoint 2	
	Mean ³	Range ⁴	Mean	Range	Mean	Range	Mean	Range
Samples, n	130		121		10		10	
Dry matter	89.57 ± 0.77	86.82 - 92.02	88.72 ± 0.57	87.02 - 90.39	95.66 ± 0.63	93.89 - 96.38	93.01 ± 2.15	90.30 - 95.37
Crude protein	47.22 ± 0.98	45.05 - 51.03	46.61 ± 0.84	44.16 - 49.41	43.02 ± 1.66	39.60 - 45.34	41.72 ± 2.99	36.36 - 46.22
Ether extract	1.86 ± 0.62	0.73 - 3.85	1.96 ± 0.62	1.07 - 5.22	9.52 ± 1.10	8.23 - 11.39	9.53 ± 0.71	8.38 - 10.60
Crude fiber	3.42 ± 0.38	2.65 - 4.31	3.44 ± 0.42	2.47 - 4.67	5.80 ± 0.24	5.52 - 6.50	5.62 ± 0.27	5.03 - 6.09
Ash	6.40 ± 0.30	5.74 - 7.13	6.19 ± 0.33	5.63 - 7.41	6.03 ± 0.23	5.69 - 6.34	5.52 ± 0.20	5.09 - 5.89
NDF	6.61 ± 0.84	4.41 - 8.93	8.00 ± 1.41	5.26 - 13.19	9.91 ± 1.40	8.40 - 13.20	9.49 ± 0.87	8.08 - 11.55

¹Five samples from each plant were collected at two timepoints within the survey: Timepoint 1 from October 2024 to January 2025 and Timepoint 2 from June to August 2025.

²All samples were analyzed in duplicate at the University of Missouri Agricultural Experiment Station Chemical Laboratory (Columbia, MO).

³Means represent the average value ± standard deviation for each individual analytical criterion within soybean meal type and timepoint of sample collection.

⁴The range is the minimum and maximum value for each individual analytical criterion within soybean meal type and timepoint of sample collection. Values represent an individual analysis for each sample submitted in duplicate.

NDF = neutral detergent fiber.

Table 2.2 Soybean meal amino acid profile by type and sampling timepoint (as-is basis).^{1,2}

Amino acid, %	Solvent extracted				Extruded-expelled			
	Timepoint 1		Timepoint 2		Timepoint 1		Timepoint 2	
	Mean ³	Range ⁴	Mean	Range	Mean	Range	Mean	Range
Samples, n	130		121		10		10	
Essential								
Arginine	3.41 ± 0.10	3.16 - 3.79	3.41 ± 0.19	3.05 - 4.09	3.13 ± 0.16	2.84 - 3.42	3.05 ± 0.22	2.62 - 3.31
Histidine	1.24 ± 0.03	1.15 - 1.36	1.25 ± 0.05	1.16 - 1.43	1.13 ± 0.05	1.05 - 1.23	1.13 ± 0.07	0.98 - 1.22
Isoleucine	2.27 ± 0.07	2.09 - 2.57	2.31 ± 0.13	2.06 - 2.74	2.07 ± 0.10	1.88 - 2.25	2.04 ± 0.02	1.80 - 2.23
Leucine	3.65 ± 0.09	3.40 - 4.04	3.68 ± 0.19	3.40 - 4.31	3.32 ± 0.14	3.09 - 3.59	3.26 ± 0.17	2.89 - 3.50
Lysine	3.05 ± 0.07	2.87 - 3.33	3.09 ± 0.15	2.85 - 3.60	2.82 ± 0.12	2.58 - 3.05	2.79 ± 0.18	2.41 - 3.00
Methionine	0.65 ± 0.02	0.60 - 0.71	0.66 ± 0.04	0.61 - 0.81	0.60 ± 0.02	0.56 - 0.62	0.59 ± 0.02	0.53 - 0.62
Phenylalanine	2.45 ± 0.07	2.27 - 2.73	2.48 ± 0.14	2.29 - 2.95	2.23 ± 0.10	2.05 - 2.41	2.19 ± 0.11	1.93 - 2.36
Threonine	1.84 ± 0.04	1.72 - 1.98	1.88 ± 0.14	1.73 - 2.38	1.70 ± 0.06	1.59 - 1.79	1.68 ± 0.11	1.45 - 1.80
Tryptophan	0.62 ± 0.03	0.38 - 0.70	0.64 ± 0.04	0.50 - 0.72	0.55 ± 0.04	0.50 - 0.64	0.56 ± 0.04	0.47 - 0.63
Valine	2.35 ± 0.07	2.16 - 2.65	2.37 ± 0.11	2.10 - 2.74	2.16 ± 0.11	1.95 - 2.36	2.12 ± 0.14	1.80 - 2.32
Non-essential								
Alanine	2.05 ± 0.05	1.19 - 2.25	2.07 ± 0.10	1.92 - 2.38	1.88 ± 0.08	1.76 - 2.02	1.85 ± 0.11	1.62 - 1.97
Aspartic Acid	5.27 ± 0.13	4.95 - 5.85	5.40 ± 0.31	4.98 - 6.50	4.84 ± 0.20	4.48 - 5.18	4.82 ± 0.31	4.15 - 5.14
Cysteine	0.69 ± 0.03	0.62 - 0.76	0.72 ± 0.06	0.62 - 0.92	0.67 ± 0.03	0.58 - 0.72	0.67 ± 0.02	0.62 - 0.71
Glutamic Acid	8.74 ± 0.23	8.18 - 9.76	8.88 ± 0.59	8.15 - 11.03	7.89 ± 0.36	7.28 - 8.54	7.80 ± 0.51	6.75 - 8.33
Glycine	2.00 ± 0.05	1.87 - 2.19	2.01 ± 0.10	1.86 - 2.37	1.88 ± 0.08	1.73 - 2.01	1.84 ± 0.13	1.58 - 1.97

Hydroxylysine	0.08 ± 0.02	0.00 - 0.14	0.06 ± 0.01	0.03 - 0.14	0.09 ± 0.01	0.07 - 0.10	0.06 ± 0.01	0.04 - 0.08
Hydroxyproline	0.07 ± 0.03	0.00 - 0.19	0.06 ± 0.02	0.03 - 0.18	0.10 ± 0.02	0.06 - 0.13	0.10 ± 0.02	0.08 - 0.13
Ornithine	0.03 ± 0.01	0.00 - 0.15	0.03 ± 0.01	0.00 - 0.12	0.03 ± 0.00	0.03 - 0.04	0.03 ± 0.01	0.02 - 0.07
Proline	2.56 ± 0.12	2.27 - 3.05	2.43 ± 0.12	2.19 - 2.71	2.31 ± 0.10	2.17 - 2.57	2.18 ± 0.17	1.84 - 2.36
Serine	2.08 ± 0.08	1.90 - 2.51	2.14 ± 0.20	1.91 - 3.06	1.94 ± 0.06	1.85 - 2.04	1.89 ± 0.13	1.64 - 2.05
Taurine	0.15 ± 0.02	0.05 - 0.18	0.13 ± 0.02	0.08 - 0.16	0.17 ± 0.01	0.15 - 0.19	0.14 ± 0.02	0.11 - 0.16
Tyrosine	1.77 ± 0.06	1.54 - 1.93	1.71 ± 0.12	1.42 - 2.09	1.65 ± 0.06	1.54 - 1.76	1.52 ± 0.08	1.36 - 1.64

¹Five samples from each plant were collected at two timepoints within the survey: Timepoint 1 from October 2024 to January 2025 and Timepoint 2 from June to August 2025.

²All samples were analyzed in duplicate at the University of Missouri Agricultural Experiment Station Chemical Laboratory (Columbia, MO).

³Means represent the average value ± standard deviation for each individual analytical criterion within soybean meal type and timepoint of sample collection.

⁴The range is the minimum and maximum value for each individual analytical criterion within soybean meal type and timepoint of sample collection. Values represent an individual analysis for each sample submitted in duplicate.

Table 2.3 Soybean meal fatty acid profile by type and sampling timepoint (as-is basis).^{1,2}

Item	Solvent extracted				Extruded-expelled			
	Timepoint 1		Timepoint 2		Timepoint 1		Timepoint 2	
	Mean ³	Range ⁴	Mean	Range	Mean	Range	Mean	Range
Samples, n	130		121		10		10	
SFA, % ⁵								
C14:0	0.12 ± 0.07	0.07 - 0.43	0.09 ± 0.02	0.07 - 0.22	0.08 ± 0.01	0.07 - 0.12	0.08 ± 0.01	0.07 - 0.09
C15:0	0.06 ± 0.03	0.00 - 0.17	0.05 ± 0.01	0.00 - 0.08	0.03 ± 0.01	0.02 - 0.04	0.03 ± 0.01	0.02 - 0.03
C16:0	15.33 ± 1.26	8.44 - 18.08	15.79 ± 0.80	12.86 - 18.14	11.48 ± 0.16	11.30 - 11.95	11.54 ± 0.46	10.89 - 12.44
C17:0	0.18 ± 0.08	0.05 - 0.64	0.15 ± 0.01	0.10 - 0.19	0.11 ± 0.02	0.02 - 0.14	0.12 ± 0.01	0.11 - 0.13
C18:0	4.41 ± 0.31	3.53 - 5.19	4.33 ± 0.24	3.62 - 4.90	4.66 ± 0.44	4.25 - 5.57	4.67 ± 0.39	4.22 - 5.30
C20:0	0.24 ± 0.03	0.18 - 0.35	0.23 ± 0.03	0.16 - 0.40	0.33 ± 0.03	0.30 - 0.39	0.33 ± 0.03	0.26 - 0.39
C22:0	0.43 ± 0.08	0.27 - 0.84	0.39 ± 0.04	0.15 - 0.70	0.38 ± 0.03	0.35 - 0.44	0.35 ± 0.03	0.26 - 0.39
C24:0	0.28 ± 0.04	0.13 - 0.46	0.23 ± 0.05	0.00 - 0.35	0.17 ± 0.02	0.15 - 0.22	0.16 ± 0.02	0.12 - 0.19
Total SFA	21.25 ± 1.40	13.84 - 26.04	21.39 ± 0.81	18.62 - 23.56	17.34 ± 0.54	16.76 - 18.43	17.37 ± 0.33	16.78 - 17.92
MUFA, % ⁵								
C16:1	0.39 ± 0.30	0.08 - 1.36	0.10 ± 0.02	0.07 - 0.24	0.17 ± 0.07	0.09 - 0.26	0.09 ± 0.01	0.08 - 0.10
C17:1	0.04 ± 0.10	0.00 - 0.86	0.04 ± 0.02	0.00 - 0.10	0.03 ± 0.02	0.01 - 0.06	0.05 ± 0.01	0.05 - 0.06
C18:1 ⁶	13.89 ± 6.76	9.70 - 60.60	12.34 ± 1.65	10.29 - 21.24	18.79 ± 0.54	18.17 - 20.08	18.83 ± 0.58	18.22 - 20.00
C20:1	0.13 ± 0.05	0.07 - 0.30	0.10 ± 0.02	0.05 - 0.26	0.17 ± 0.01	0.15 - 0.17	0.15 ± 0.04	0.02 - 0.17
C24:1	0.07 ± 0.13	0.00 - 0.69	0.03 ± 0.04	0.00 - 0.15	0.03 ± 0.03	0.02 - 0.15	0.02 ± 0.01	0.00 - 0.04
Total MUFA	16.01 ± 6.87	11.39 - 63.24	14.05 ± 1.68	11.84 - 23.09	20.72 ± 0.57	20.05 - 21.97	20.62 ± 0.62	19.93 - 21.79

PUFA, %⁵

C18:2 ⁶	51.77 ± 5.53	17.05 - 56.92	53.39 ± 1.26	45.49 - 56.24	53.19 ± 0.91	51.57 - 54.17	53.28 ± 0.65	52.26 - 54.30
C18:3 ⁶	7.95 ± 1.04	3.80 - 11.21	8.06 ± 0.71	6.55 - 10.33	7.60 ± 0.27	7.07 - 7.91	7.54 ± 0.29	6.98 - 7.95
C20:5	0.07 ± 0.09	0.00 - 0.42	0.01 ± 0.03	0.00 - 0.13	0.01 ± 0.02	0.00 - 0.08	0.00	0.00 - 0.00
Total PUFA	60.13 ± 6.00	20.89 - 68.34	61.50 ± 1.44	52.46 - 64.68	60.94 ± 1.04	59.27 - 62.17	60.89 ± 0.92	59.31 - 62.29

¹Five samples from each plant were collected at two timepoints within the survey: Timepoint 1 from October 2024 to January 2025 and Timepoint 2 from June to August 2025.

²All samples were analyzed in duplicate at the University of Missouri Agricultural Experiment Station Chemical Laboratory (Columbia, MO).

³Means represent the average value ± standard deviation for each individual analytical criterion within soybean meal type and timepoint of sample collection.

⁴The range is the minimum and maximum value for each individual analytical criterion within soybean meal type and timepoint of sample collection. Values represent an individual analysis for each sample submitted in duplicate.

⁵Presented as a percentage of extracted lipid.

⁶Concentration includes isomers.

MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SFA = saturated fatty acids.

Table 2.4 Soybean meal mineral analysis by type and sampling timepoint (as-is basis).^{1,2}

Item	Solvent extracted				Extruded-expelled			
	Timepoint 1		Timepoint 2		Timepoint 1		Timepoint 2	
	Mean ³	Range ⁴	Mean	Range	Mean	Range	Mean	Range
Samples, n	130		121		10		10	
Macrominerals, %								
Ca	0.39 ± 0.13	0.21 - 0.75	0.39 ± 0.14	0.18 - 0.84	0.28 ± 0.02	0.25 - 0.31	0.27 ± 0.04	0.21 - 0.33
K	2.22 ± 0.14	1.37 - 2.50	2.30 ± 0.12	1.87 – 2.60	2.10 ± 0.10	1.88 - 2.24	2.09 ± 0.14	1.86 - 2.33
Mg	0.25 ± 0.01	0.22 - 0.30	0.27 ± 0.02	0.21 - 0.33	0.24 ± 0.01	0.22 - 0.26	0.24 ± 0.02	0.22 - 0.28
Na	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
P	0.63 ± 0.04	0.54 - 0.73	0.66 ± 0.04	0.53 – 0.77	0.57 ± 0.02	0.53 - 0.60	0.54 ± 0.06	0.44 - 0.64
Microminerals, mg/kg								
Cu	14.20 ± 1.44	10.50 - 22.90	11.94 ± 1.59	9.01 - 18.60	12.54 ± 1.31	10.60 - 15.20	11.18 ± 2.64	8.22 - 17.80
Fe	103.69 ± 21.20	70.00 - 196.00	95.11 ± 23.39	61.20 - 219.20	119.05 ± 15.64	85.00 - 152.00	91.06 ± 7.43	77.40 - 104.00
Mn	34.72 ± 4.73	23.90 - 48.20	47.64 ± 4.53	35.50 – 65.20	32.73 ± 6.35	24.00 - 44.10	29.35 ± 3.97	22.30 - 39.20
Zn	58.01 ± 6.07	45.70 - 76.70	34.29 ± 5.04	23.10 – 45.40	54.29 ± 6.54	43.00 - 62.90	45.05 ± 5.58	38.00 - 50.80

¹Five samples from each plant were collected at two timepoints within the survey: Timepoint 1 from October 2024 to January 2025 and Timepoint 2 from June to August 2025.

²All samples were analyzed in duplicate at the University of Missouri Agricultural Experiment Station Chemical Laboratory (Columbia, MO).

³Means represent the average value ± standard deviation for each individual analytical criterion within soybean meal type and timepoint of sample collection.

⁴The range is the minimum and maximum value for each individual analytical criterion within soybean meal type and timepoint of sample collection. Values represent an individual analysis for each sample submitted in duplicate.

Table 2.5 Soybean meal carbohydrate analysis by type and sampling timepoint (as-is basis).^{1,2}

Item, %	Solvent extracted				Extruded-expelled			
	Timepoint 1		Timepoint 2 ³		Timepoint 1		Timepoint 2	
	Mean ⁴	Range ⁵	Mean	Range	Mean	Range	Mean	Range
Samples, n	130		25		10		4	
Sucrose	6.47 ± 0.81	3.88 - 8.70	6.87 ± 0.54	5.16 - 8.00	6.30 ± 0.86	5.12 - 8.06	5.76 ± 0.53	5.34 - 6.26
Stachyose	5.27 ± 0.71	3.96 - 8.56	4.89 ± 1.21	1.35 - 7.53	5.37 ± 0.73	4.26 - 6.67	4.40 ± 1.07	3.59 - 5.45
Raffinose	1.03 ± 0.16	0.65 - 1.81	1.23 ± 0.20	0.73 - 1.72	1.10 ± 0.32	0.36 - 1.51	1.20 ± 0.22	1.01 - 1.39
Verbascose	0.17 ± 0.05	0.05 - 0.32	0.11 ± 0.06	0.00 - 0.26	0.17 ± 0.07	0.06 - 0.30	0.11 ± 0.08	0.06 - 0.19

¹Five samples from each plant were collected at two timepoints within the survey: Timepoint 1 from October 2024 to January 2025 and Timepoint 2 from June to August 2025.

²All samples were analyzed in duplicate at the University of Missouri Agricultural Experiment Station Chemical Laboratory (Columbia, MO).

³One sample per plant was analyzed in timepoint 2.

⁴Means represent the average value ± standard deviation for each individual analytical criterion within soybean meal type and timepoint of sample collection.

⁵The range is the minimum and maximum value for each individual analytical criterion within soybean meal type and timepoint of sample collection. Values represent an individual analysis for each sample submitted in duplicate.

Table 2.6 Soybean meal quality characteristics by type and sampling timepoint (as-is basis).¹

Item ²	Solvent extracted				Extruded-expelled			
	Timepoint 1		Timepoint 2		Timepoint 1		Timepoint 2	
	Mean ³	Range ⁴	Mean	Range	Mean	Range	Mean	Range
Samples, n	130		121		10		10	
TIU/mg	4.34 ± 0.45	0.61 - 7.97	4.85 ± 0.43	1.98 - 9.58	14.91 ± 1.35	7.84 - 20.68	14.57 ± 1.55	9.61 - 19.90
UA, Δ pH ⁵	0.22 ± 0.28	0.03 - 1.23	0.29 ± 0.48	0.03 - 2.20	0.86 ± 0.84	0.12 - 1.70	1.02 ± 0.55	0.45 - 1.56
PDI, %	25.28 ± 6.89	14.05 - 48.37	16.43 ± 3.86	8.60 - 28.44	18.76 ± 6.83	9.11 - 27.29	15.10 ± 3.05	9.37 - 20.40
KOH, % ⁵	87.50 ± 2.75	82.53 - 93.65	85.53 ± 4.33	74.82 - 92.76	84.50 ± 2.96	80.63 - 87.34	88.25 ± 1.12	86.63 - 89.05

¹Five samples from each plant were collected at two timepoints within the survey: Timepoint 1 from October 2024 to January 2025 and Timepoint 2 from June to August 2025.

²All samples were analyzed in duplicate at the University of Missouri Agricultural Experiment Station Chemical Laboratory (Columbia, MO) besides TIU, which was analyzed in triplicate at Agricultural Research Service, US Department of Agriculture (Colombia, MO).

³Means represent the average value ± standard deviation for each individual analytical criterion within soybean meal type and timepoint of sample collection.

⁴The range is the minimum and maximum value for each individual analytical criterion within soybean meal type and timepoint of sample collection. Values represent an individual analysis for each sample submitted in duplicate.

⁵One sample per plant was analyzed. Solvent extracted timepoint 1, n = 26; solvent extracted timepoint 2, n = 24; extruded-expelled, n = 2.

KOH = protein solubility in 0.2% potassium hydroxide; PDI = protein dispersibility index; TIU = trypsin inhibitor units; UA = urease activity.

Table 2.7 Soybean meal physical characteristics by type and sampling timepoint (as-is basis).^{1,2}

Item	Solvent extracted				Extruded-expelled			
	Timepoint 1		Timepoint 2		Timepoint 1		Timepoint 2	
	Mean ³	Range ⁴	Mean	Range	Mean	Range	Mean	Range
Samples, n	130		121		10		10	
Bulk density, g/cm ³	0.64 ± 0.02	0.59 - 0.71	0.62 ± 0.03	0.57 - 0.75	0.56 ± 0.04	0.51 - 0.60	0.58 ± 0.02	0.55 - 0.60
Angle of repose, °	40.04 ± 1.51	36.67 - 42.86	41.20 ± 1.14	38.5 - 44.1	46.03 ± 0.52	45.17 - 46.69	43.70 ± 0.50	42.64 - 44.42
Particle size, µm	925 ± 188	614 - 1,508	863 ± 154	546 - 1,336	551 ± 216	335 - 832	798 ± 109	541 - 927

¹Five samples from each plant were collected at two timepoints within the survey: Timepoint 1 from October 2024 to January 2025 and Timepoint 2 from June to August 2025.

²Samples were analyzed at the Kansas State University Swine Laboratory.

³Means represent the average value ± standard deviation for each individual analytical criterion within soybean meal type and timepoint of sample collection.

⁴The range is the minimum and maximum value for each individual analytical criterion within soybean meal type and timepoint of sample collection. Values represent an individual analysis for each sample submitted in duplicate.

Chapter 3 - Analytical variation between and within laboratories when measuring US soybean meal composition and quality

Abstract

Accurate laboratory analysis is critical for evaluating soybean meal composition and quality for diet formulation. Therefore, the objective of this study was to quantify between and within laboratory variation for common soybean meal analytical assays. A 2.25 kg sample of soybean meal was collected from 27 US soybean processing plants and analyzed in quadruplicate for proximate analysis, neutral detergent fiber (NDF), Ca, P, trypsin inhibitor (TI), and amino acid profile. Samples were analyzed at four laboratories except for trypsin inhibitor which was analyzed at three. Data are presented through descriptive statistics as mean $\pm$ standard deviation, with corresponding minimum and maximum values to describe the range for each analysis within laboratory. To further evaluate analytical variation, the coefficient of variation (CV) was calculated for each analyte. Within laboratory CV was calculated using the mean and standard deviation of the four independent subsamples analyzed within each laboratory, representing analytical repeatability. Between laboratory CV was calculated using the mean values from each laboratory for a given analyte, with the standard deviation representing variation between laboratories, reflecting analytical reproducibility. Within laboratory variation indicated strong repeatability for most analyses, with dry matter and crude protein consistently below 3.54% CV and the majority of samples compared within labs had below a 10% CV across assays. In contrast, TI exhibited the greatest variability with CV values ranging from 4.67 to 62.09% in one lab, and direct comparison between laboratories was not appropriate due to methodological differences. Between laboratory variation was lowest for dry matter and crude

protein, with CV values ranging from 0.33 to 0.76% and 0.05 to 1.49%, respectively. Moderate variation was observed for some analytes with CV values ranging from 1.84 to 12.92% for crude fiber, 2.55 to 9.64% for ash, 0.93 to 15.45% for Ca, and 1.22 to 6.87% for P, whereas ether extract and NDF had the greatest variation with CV values ranging from 1.52 to 32.67% and 2.03 to 23.07%, respectively. Amino acid variability was analyte-dependent, ranging from 0.25 to 31.25%. Quantifying analytical variation between and within laboratories provides insight into the repeatability and reproducibility of commonly used soybean meal assays and will help guide best practices when evaluating soybean meal for diet formulation.

Key Words: analytical variation, coefficient of variation, repeatability, reproducibility, soybean meal quality, trypsin inhibitor

Introduction

Accurate laboratory analysis of feed ingredients is essential for precise diet formulation, ingredient evaluation, and quality control. However, reported analytical values may vary between laboratories and among replicate analyses conducted within the same laboratory. This variation may result from differences in sample preparation, subsampling, analytical procedures, instrumentation, calibration, technician technique, or laboratory specific modifications to established methods. Soybean meal is commonly evaluated using multiple analytical criteria; therefore, understanding the repeatability and reproducibility of these assays is important when interpreting reported nutrient composition and quality measurements.

Previous research has demonstrated that laboratory variation can meaningfully influence reported nutrient values. Cromwell et al. (1999) evaluated corn and soybean meal samples across 22 laboratories and reported that, except for crude protein and selenium, variation between

laboratories was as great as or greater than variation among ingredient sources. Within laboratory variation was generally lower than between laboratory variation, indicating that laboratory selection can affect reported values. Similarly, Hristov et al. (2010) observed considerable between laboratory variation in neutral detergent fiber analysis of feed ingredients and complete diets, whereas crude protein results were more consistent. These findings demonstrate the need to consider analytical variation independently for each analysis commonly used for soybean meal.

Analytical variation can be evaluated in terms of repeatability and reproducibility. Repeatability describes the consistency of results when replicate subsamples are analyzed within the same laboratory, whereas reproducibility describes the consistency of results when identical samples are analyzed by different laboratories. The coefficient of variation is commonly used to express analytical variability because it standardizes the standard deviation relative to the mean, allowing variation to be compared among assays with different mean concentrations when evaluating repeatability (Reed et al., 2002). However, when using CV to evaluate analytical variability associated with reproducibility, the measurement is still sensitive to differences in reporting units. Evaluating both repeatability and reproducibility provides a practical framework for determining whether differences in reported values may reflect analytical variation rather than true differences among samples.

Although previous studies have evaluated laboratory variation in feed analyses, much of this work was conducted more than 15 yr ago, included multiple feed ingredients, or evaluated a limited number of analytical criteria. Updated information is needed to characterize within and between laboratory variation for assays commonly used to evaluate US soybean meal, including proximate composition, fiber, minerals, amino acids, and processing quality indicators.

Quantifying potential variation provides practical information for interpreting soybean meal analytical results and identifying assays where laboratory selection or method standardization may be especially important. Therefore, the objective of this study was to evaluate within and between laboratory variation for commonly used soybean meal analytical procedures by submitting subsamples of soybean meal in quadruplicate to multiple commercial laboratories.

Materials and methods

The soybean meal samples used in this study were collected as part of an industry survey designed to characterize the composition and quality of US soybean meal (Collier et al., 2026). A representative sample (2.25 kg) was collected from 27 different US soybean processing plants between June and August 2025. Of the 27 plants, 25 were solvent extracted and 2 plants were mechanically extruded-expelled.

Each plant received pre-labeled sampling bags (Whirl-Pak, Nasco, Ft. Atkinson, WI) prior to the time of collection. Upon receipt of the sampling packages, an employee from each soybean processing plant was instructed to collect a representative sample from that processing day. Information regarding the date of collection and whether a soybean oil refinery is located adjacent to the plant was recorded. Once collected, the samples were shipped to Kansas State University. All samples were stored at room temperature until analyses were performed. Each sample was homogenized through a riffle splitter then ground to approximately 220 μm . Ground samples were then divided into 16 subsamples to be analyzed individually, such that each laboratory received four subsamples per original sample. In total, each laboratory received 108 samples (4 subsamples from 27 plants). Laboratories were blinded to samples received.

Each sample was analyzed for proximate analysis, amino acid profile, neutral detergent fiber, Ca, P, and trypsin inhibitor. These measurements were selected because they represent key

elements of soybean meal composition for diet formulation and are frequently used to define soybean meal quality. Samples were analyzed at four laboratories: University of Missouri (Columbia, MO), Eurofins Food Chemistry Testing Center (Des Moines, IA), ATC Scientific (Little Rock, AR), and Midwest Laboratories (Omaha, NE), except for trypsin inhibitor which was analyzed at USDA-ARS (Columbia, MO), Eurofins Food Chemistry Testing Center, and ATC Scientific. These laboratories were selected as they are widely used commercial labs across the animal feed industry and offer a breadth of testing procedures. Inclusion in this study does not imply endorsement of these specific commercial laboratories.

Chemical analysis

At ATC Scientific (Little Rock, AR), proximate composition was determined including dry matter (Method Ba 2a-38; AOCS), crude protein (Method Ba 4f-00; AOCS), ether extract (Method Ba 3-38; AOCS), crude fiber (Method Ba 6a-05; AOCS), and ash (Method Ba 5a-49; AOCS), as well as neutral detergent fiber (ANKOM 6 Technology). Complete amino acid profile (Method 994.12, 988.15, 982.30; AOAC), mineral analysis by inductively plasma-atomic emission spectrometry (Method 2011.14; AOAC) and trypsin inhibitor (Method 14902; ISO, 2001) were also determined.

At Eurofins Food Chemistry Testing Center (Des Moines, IA), proximate composition was determined including dry matter (Method Ba 2a-38; AOCS), crude protein (Method 990.03; AOAC), ether extract (Method Ba 3-38; AOCS), crude fiber (Method 962.09; AOAC), and ash (Method 942.05; AOAC), as well as neutral detergent fiber (A2000 ANKOM Technology). Complete amino acid profile (Method 982.30; AOAC), mineral analysis by inductively coupled plasma optical emission spectroscopy (Method 985.01; AOAC), and trypsin inhibitor (Ba 12a-2020; AOCS) were also determined.

At Midwest Laboratories (Omaha, NE), proximate composition was determined including dry matter (Method 930.15; AOAC), crude protein (Method 990.03; AOAC), ether extract (Method 2003.05; AOAC), crude fiber (Method Ba 6a-05; AOCS), and ash (Method 942.05; AOAC), as well as neutral detergent fiber (ANKOM technology). Complete amino acid profile (Method 994.12, 988.15; AOAC) and mineral analysis by inductively coupled plasma optical emission spectroscopy (Method 985.01; AOAC) were also determined.

At the University of Missouri (Columbia, MO), proximate composition was determined including dry matter (Method 934.01; AOAC, 2006), crude protein (Method 990.03; AOAC, 2006), ether extract (Method 920.39; AOAC, 1998), crude fiber (Method 978.10, AOAC, 2006), and ash (Method 942.05; AOAC, 2005), as well as neutral detergent fiber (ANKOM Technology, 2017). Complete amino acid profile (Method 982.30 E (a, b, c), 988.15; AOAC, 2006), and mineral analysis by inductively coupled plasma optical emission spectroscopy (Method 985.01 A, B, and D; AOAC) were also determined. Each soybean meal sample was submitted for analysis of trypsin inhibitor units in triplicate at USDA-ARS (Columbia, MO) utilizing procedures outlined by Kim and Krishnan (2023).

Statistical analysis

Descriptive statistics were used to visualize means and standard deviation of each analytical criteria within soybean meal type and laboratory analyzed. Additionally, the range was included, which represented the minimum and maximum values for each analytical criteria within the soybean meal type and laboratory. The range was based on an individual analysis and does not represent the values of a sample analyzed in quadruplicate. Mean and range values were presented separately for solvent extracted and extruded-expelled soybean meal to describe composition by processing method. However, CV values were summarized across soybean meal

types because analytical variation appeared to fall within similar ranges, although comparisons between processing methods were limited by the small number of extruded-exelled processing plants ($n = 2$). To further evaluate analytical variation, the coefficient of variation (CV) was calculated for each analyte. Within laboratory CV was calculated using the mean and standard deviation of the four independent subsamples analyzed within each laboratory, representing analytical repeatability. Between laboratory CV was calculated using the mean values from each laboratory for a given analyte, with the standard deviation representing variation between laboratories, reflecting analytical reproducibility.

Results and discussion

Mean $\pm$ standard deviation and ranges for each analysis by laboratory are presented in Tables 1 to 5, while between laboratory variation (Figures 1 to 3) and within lab variation (Figures 4 and 5) are illustrated using CV percentage. Together, these metrics provide complementary insight into the repeatability and reproducibility of reported values across laboratories and indicate that analytical variability was assay dependent. Results are discussed by analytical category, with emphasis on both between laboratory reproducibility and within laboratory repeatability.

For interpretation, CV was used as a standardized measure of analytical variation because it expresses the standard deviation relative to the mean, allowing comparisons across assays with different concentrations when evaluating repeatability (Reed et al., 2002). However, when using CV to evaluate analytical variability associated with reproducibility, the measurement is still sensitive to differences in reporting units. In applied assay validation, CV thresholds are commonly used to describe analytical precision, with values below 15 to 20% often considered acceptable depending on the assay and intended use (Bansal and DeStefano, 2007). Therefore, in

the present study, CV values less than 5% were interpreted as low variation, values between 5 and 10% as moderate variation, values greater than 10% as elevated variation, and values greater than 20% as highly variable. These categories were used to describe relative differences among assays, rather than to define whether a specific laboratory result was acceptable or unacceptable. This distinction is important because a high CV among replicate analyses within the same laboratory indicates limited repeatability, whereas a high CV between laboratories may reflect differences in sample preparation, analytical procedures, equipment, calibration, or other laboratory specific factors (Horwitz, 1982; Horwitz et al., 1998).

Proximate composition

Dry matter and crude protein were the most consistent proximate analyses evaluated in this study. Across solvent extracted and extruded-expelled soybean meal samples, between laboratory CV ranged from 0.33 to 0.76% for dry matter and from 0.05 to 1.49% for crude protein. Within laboratory variation was also low, with maximum CV values of 0.98% for dry matter and 3.54% for crude protein. These results indicate strong repeatability within laboratories and reproducibility between laboratories for these analyses. The low variation observed for dry matter was expected, as the analysis is relatively simple and requires limited sample manipulation compared with more complex extraction or residue-based assays. Crude protein analysis is also highly standardized across commercial laboratories, which likely contributed to the low variation observed in the present study. These findings are consistent with those of Cromwell et al. (1999), who observed that crude protein was among the least variable nutrient analyses when corn and soybean meal samples were evaluated across laboratories. Similarly, Hristov et al. (2010) observed greater consistency for crude protein than neutral detergent fiber in an evaluation of laboratory variation of individual ingredients and complete diets.

In contrast to dry matter and crude protein, ether extract exhibited greater analytical variation. Between laboratory CV for ether extract ranged from 1.52 to 32.67%, indicating less consistent reproducibility between laboratories for this assay. Although all laboratories quantified ether extract using solvent based extraction procedures, the specific methods differed between laboratories. In the present study, ether extract was analyzed using AOCS Ba 3-38 at two laboratories, AOAC 920.39 at one laboratory, and AOAC 2003.05 at one laboratory. The AOCS Ba 3-38 is specifically intended for the analysis of residual oil in oilseed meals and uses an extended solvent extraction procedure, whereas AOAC 920.39 is a broader crude fat method that uses conventional Soxhlet extraction, in which condensed solvent repeatedly passes through the sample over an extended extraction period. In contrast, AOAC 2003.05 uses a two-step Randall/Soxtec procedure in which the sample is first submerged in boiling solvent and then rinsed with condensed solvent, reducing the time required for extraction (Thiex et al., 2003). Ether extract is influenced by differences in solvent exposure, extraction temperature, and extraction duration may affect the amount and types of ether soluble material recovered (Palmquist and Jenkins, 2003). Previous method comparisons have also reported differences in measured ether extract values even when the same solvent was used, with the magnitude of the difference varying among sample types and potentially reflecting differences in the extraction of non-fatty compounds (Silva et al., 2011; Barbosa et al., 2017). This is relevant for solvent extracted soybean meal because ether extract concentrations are low; therefore, small numerical differences between laboratories can represent a relatively large percentage of the mean and result in elevated CV values. Within laboratory variation for ether extract was also greater than observed for dry matter and crude protein, with CV values averaging 6.61 ± 3.94 for Lab A, 5.54 ± 4.47 for Lab B, 7.47 ± 5.91 for Lab C, and 6.82 ± 3.47 for Lab D. Where CV values ranged

from 0.32 to 30.27%; however, 85% of the samples remained below 10% CV, suggesting that repeatability was acceptable for most of the samples analyzed.

Neutral detergent fiber

Neutral detergent fiber (NDF) also exhibited relatively high analytical variation, where between laboratory CV ranged from 2.03 to 23.07%. Although NDF was analyzed using generally similar ANKOM-based procedures across laboratories, use of the same analytical platform does not ensure that the procedures were implemented identically. Neutral detergent fiber is an empirical, gravimetric assay, meaning that the reported value depends on the specific extraction, filtration, washing, drying, and residue-recovery conditions used. Sample preparation and grinding were standardized before samples were distributed, minimizing their contribution to the observed between laboratory variation. However, differences among laboratories in detergent preparation and extraction, heat-stable α -amylase source and concentration, sodium sulfite use, filter bag handling, rinsing, drying, and residue recovery may affect the amount of residue measured and the resulting NDF concentration (Mertens, 2002; Fahey et al., 2019; Hall and Mertens, 2023). Among those factors, filtration technique and contamination of the recovered residue with starch or protein have been identified as major sources of variation in NDF analysis (Mertens, 1992). Heat-stable α -amylase is used to remove starch and facilitate filtration, whereas sodium sulfite reduces protein contamination of the recovered residue, particularly in heated feed ingredients (Ferreira and Mertens, 2007; Ferreira and Thiex, 2023). Consequently, differences in the application of these reagents and recovery of the final residue may produce discrepancies even when similar ANKOM-based procedures are used. Within laboratory variation for NDF was greater than observed for dry matter and crude protein, with CV values ranging from 0.67 to 13.25% for Lab A, 1.83 to 13.73% for Lab B, 1.25 to 9.56% for Lab C, and 2.71 to 22.06% for

Lab D. Overall, 80% of the samples were below a 10% CV. The upper end of this range was largely driven by one laboratory, suggesting that small procedural differences within an analytical run or laboratory workflow could have influenced NDF results. The greater variation observed for NDF in the present study is consistent with Hristov et al. (2010), who observed greater between laboratory variation for NDF than crude protein in feed ingredients and complete diet samples.

Mineral composition

Calcium demonstrated moderate analytical variation, where between laboratory CV ranged from 0.93 to 15.45% and within laboratory CV ranged from 0.21 to 33.85%. However, 81% of samples were below a CV of 10% for both between and within laboratory comparisons, suggesting acceptable reproducibility between laboratories and repeatability within laboratories for Ca analysis for most samples. Although laboratories used generally similar plasma based mineral analysis procedures, the upper end of these ranges could reflect slight differences in sample preparation and digestion, dilution, calibration, and ICP operating conditions, rather than consistently poor reproducibility or repeatability. As CV values within each lab averaged 6.19 ± 4.44 for Lab A, 4.26 ± 4.48 for Lab B, 7.06 ± 6.90 for Lab C, and 7.24 ± 5.40 for Lab D. Phosphorus exhibited low analytical variation, where between laboratory CV ranged from 1.22 to 6.87%. Within laboratory variation for P ranged from 0.00 to 12.72%, with 90% of samples below a CV of 5%, indicating strong repeatability within laboratories for most samples. Overall, these results suggest that Ca and P analyses were generally reproducible between laboratories and repeatable within laboratories in the present study.

Aforementioned, all soybean meal samples were ground using a standardized procedure before being shipped to participating laboratories, which likely minimized variation associated

with initial particle size and sample heterogeneity. However, standardized grinding does not ensure that individual minerals are distributed equally throughout each analytical portion. Cromwell et al. (1999) observed greater laboratory variation for Ca than P despite finely grinding and subdividing corn and soybean meal samples before distribution. This difference may be related to how uniformly Ca and P are distributed throughout the sample because P is primarily present as an intrinsic component of soybean meal, whereas Ca may include both intrinsic and added limestone as a flow agent. Limestone is commonly added to soybean meal during handling and storage as a flow agent to improve product flowability, with allowable inclusion rates of up to 0.5% (Batal et al., 2010; NOPA, 2025). Unlike intrinsic minerals that are distributed within the soybean tissue, added limestone may not be distributed uniformly throughout the meal, potentially increasing variation among analytical subsamples. Thiex and Ramsey (2023) demonstrated that Ca added as calcium carbonate exhibited substantially greater subsampling variation than P naturally present within feed ingredients and that grinding the entire sample before subsampling markedly reduced, but did not eliminate, Ca variation. Therefore, the greater variation observed for Ca than P in the present study may be related to differences in how uniformly the minerals were distributed throughout the ground sample, as well as variation introduced during laboratory test-portion selection, subsampling, and analysis.

Amino acid profile

Amino acid profile analysis demonstrated low to moderate analytical variation, although the magnitude of variation depended on the specific amino acid evaluated. Among essential amino acids, histidine and lysine were among the most consistent, with between laboratory CV values remaining below 6%. In contrast, isoleucine, tryptophan, and valine exhibited greater between laboratory variability, with maximum CV values exceeding 10%. However, more than

277 80% of samples for these amino acids were below the CV of 10%, indicating acceptable
278 reproducibility between laboratories can be achieved. As expected, tryptophan exhibited the
279 greatest range in CVs for between laboratory variation among essential amino acids, with CV
280 values ranging from 0.95 to 15.13%. This increased variability of tryptophan is consistent with
281 the requirement for alkaline hydrolysis, which differs from the standard acid hydrolysis used for
282 most amino acids. Methionine and tryptophan were not analyzed across all laboratories, as they
283 were not included in standard amino acid packages at Lab B. Within laboratory CV values for
284 essential amino acids were generally low (data not shown), indicating acceptable repeatability
285 within laboratories. Although occasional elevated values were observed, the average within
286 laboratory CV across essential amino acid analyses was 3.31%. Isoleucine and valine had
287 moderate within laboratory variation, with CV values ranging from 0 to 11.32% and 0.22 to
288 23.82%, respectively. Differences in variation among amino acids may be partially related to
289 amino acid specific responses during sample hydrolysis. Valine and isoleucine are released
290 relatively slowly during acid hydrolysis, whereas serine and threonine may be progressively
291 degraded and tyrosine is susceptible to oxidation (Rees, 1946; Gehrke et al., 1985; Rowan et al.,
292 1992; Albin et al., 2000). Within laboratory, small differences in hydrolysis time, temperature,
293 acid concentration, or sample handling may disproportionately affect amino acids that are slowly
294 released or susceptible to degradation, thereby increasing variation among replicate analyses
295 (Gehrke et al., 1985; Rowan et al., 1992; Albin et al., 2000). However, lysine had relatively low
296 within laboratory variation, with CV values averaging 2.69%, indicating strong repeatability of
297 this analysis. Methionine and tryptophan had moderate to high within laboratory variation
298 relative to other amino acids, with methionine CV values ranging from 0.78 to 11.19% and
299 tryptophan CV values ranging from 0.68 to 16.57%. Overall, essential amino acid analyses were

generally reproducible between laboratories and repeatable within laboratories, but select amino acids were more sensitive to analytical procedure.

For nonessential amino acids, glutamic acid and proline were the most consistent, with between laboratory CV ranging from 0.74 to 5.97% and 1.00 to 5.88%, respectively. In contrast, cysteine and tyrosine had greater analytical variation, with between laboratory CV ranging from 2.37 to 31.25% for cysteine and from 5.58 to 13.55% for tyrosine. The greater variation observed for cysteine is expected because sulfur containing amino acids are more sensitive to oxidation and degradation during sample hydrolysis and preparation (MacDonald et al., 1985). The elevated maximum CV for cysteine was driven by a limited number of samples as it was not analyzed as part of the standard amino acid packages at Labs B and C, indicating that the assay was generally reproducible for most samples but more variable in specific cases. Similarly, the greater variation observed for tyrosine may be related to its susceptibility to oxidation and degradation during hydrolysis (Gehrke et al., 1985; Rowan et al., 1992; Albin et al., 2000). Within laboratory CV values for non-essential amino acids were generally low (data not shown), indicating acceptable repeatability within laboratories. Although occasional elevated values were observed, the average within laboratory CV across non-essential amino acid analyses was 3.40%. Overall, nonessential amino acid analysis was generally consistent across laboratories.

Trypsin inhibitor

Trypsin inhibitor analysis exhibited the greatest analytical variation among the assays evaluated; however, interpretation of these results differs from the other analytical categories because of differences in methodology. Laboratory A reported trypsin inhibitor activity as mg/g using ISO 14902, whereas Labs B and D reported trypsin inhibitor units per mg using AOCS Ba 12a-2020 and the procedure described by Kim and Krishnan (2023), respectively. Therefore,

between laboratory CV was not considered appropriate as the scale for reporting results differed due to the procedures, limiting the comparisons across laboratories. Mean and standard deviation values are presented by laboratory.

Within laboratory CV provided a more appropriate assessment of repeatability for trypsin inhibitor analysis. Variation was highly dependent on laboratory as within laboratory CV averaged 28.30 ± 13.57 for Lab A, 4.74 ± 3.41 for Lab B, and 10.51 ± 6.94 for Lab D. Across laboratories, approximately 51% of within laboratory CV estimates were below 10%. Lab A had the greatest within laboratory variation, with 77% of samples exceeding a CV of 20%, whereas Lab B had no samples above 20% CV and only 7% of samples above 10% CV. Lab D was intermediate, with only 4% of samples exceeding 20% CV. This pattern indicates that the high overall CV range was primarily driven by one laboratory rather than low repeatability being consistent across all laboratories. Trypsin inhibitor assays are enzymatic colorimetric procedures, and previous method comparisons have shown that differences in substrate type, trypsin standardization, and calculation approach can affect reported values (Sueiro et al., 2015; Liu, 2021; Kim and Krishnan, 2023). In particular, ISO 14902 calculates activity using an assumed trypsin enzymatic purity of 56%, whereas AOCS Ba 12a-2020 standardizes results according to the specific activity of the trypsin reagent. Thus, variation in reagent activity among preparations or analytical runs could contribute to within laboratory variation (Liu, 2021). Additionally, greater relative variation may occur when inhibitor activity is low, as demonstrated in an international collaborative evaluation of AOCS Ba 12a-2020 (Liu et al., 2021). However, the lower CV observed for Labs B and D should not be interpreted as evidence that TIU based procedures are inherently more repeatable, because analytical method and laboratory were confounded in the present study, and previous comparisons have reported similar repeatability

for ISO 14902 and AACC based procedures (Sueiro et al., 2015). These results indicate that trypsin inhibitor values should be interpreted cautiously when comparing results generated by different laboratories, as differences in analytical method, units, and within laboratory repeatability may influence conclusions about the degree of soybean meal processing.

Conclusions

Analytical variation in soybean meal composition and quality measurements was assay dependent. Dry matter and crude protein were highly repeatable within laboratories and reproducible between laboratories, whereas ether extract, NDF, select amino acids, and TI exhibited greater variation. Within laboratory variation was generally low for most analyses, suggesting acceptable repeatability of replicate subsamples within a laboratory. However, greater between laboratory variation for some assays indicates that values generated by different laboratories may not always be directly comparable. Quantifying analytical variation between and within laboratories provides insight into the repeatability and reproducibility of commonly used soybean meal assays and may help guide laboratory selection, method standardization, and interpretation of results used for ingredient evaluation, quality control, and precision diet formulation.

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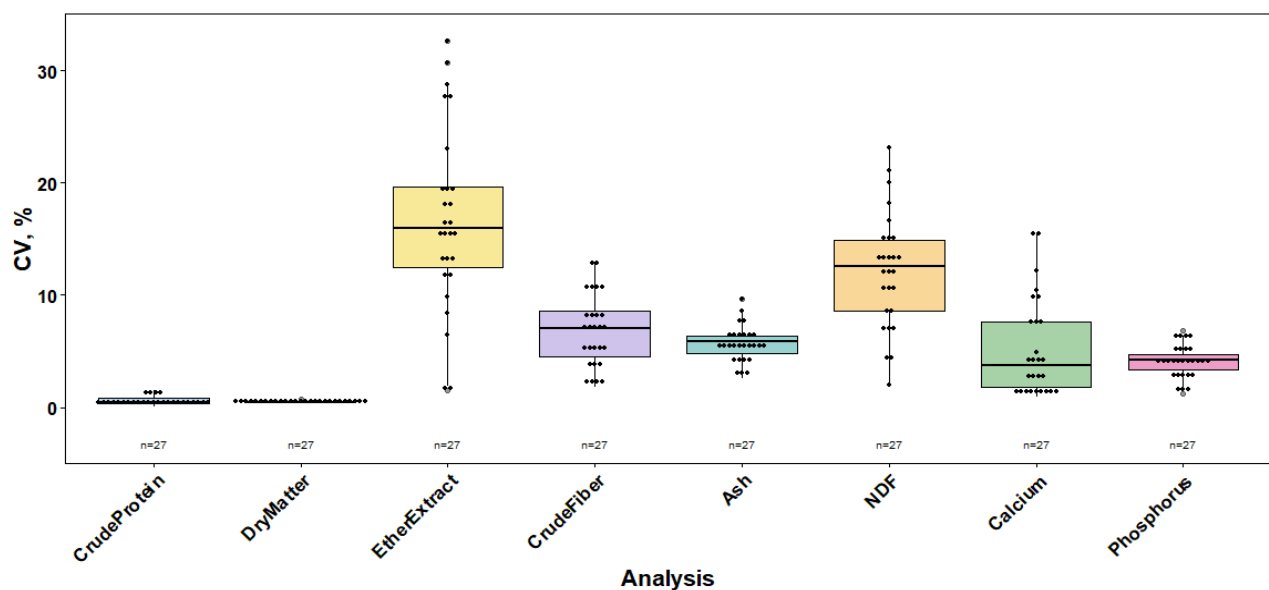


Figure 3.1 Between laboratory coefficient of variation (CV) of soybean meal composition characteristics by analysis. Individual boxplots are presented for each analysis where each data point represents the CV calculated for a single soybean meal sample across four laboratories. Between laboratory CV was calculated using the mean values from each laboratory for a given analyte, with the standard deviation representing variation between laboratories, reflecting analytical reproducibility. NDF = neutral detergent fiber.

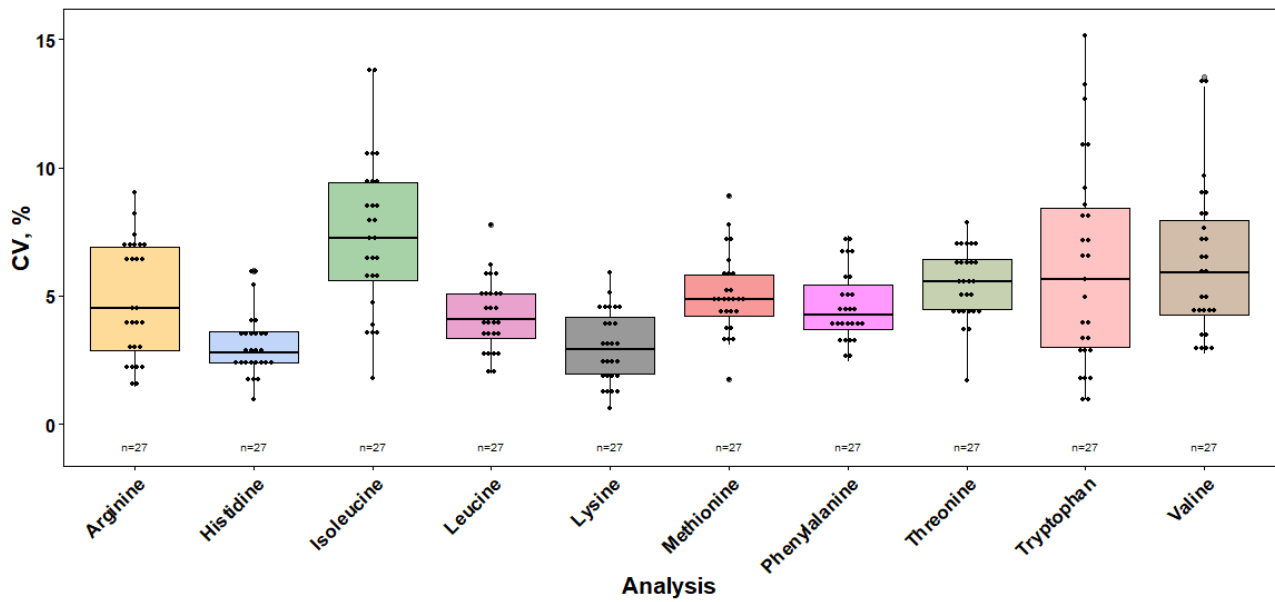


Figure 3.2 Between laboratory coefficient of variation (CV) of soybean meal essential amino acids by analysis. Individual boxplots are presented for each analysis where each data point represents the CV calculated for a single soybean meal sample across four laboratories. Between laboratory CV was calculated using the mean values from each laboratory for a given analyte, with the standard deviation representing variation between laboratories, reflecting analytical reproducibility.

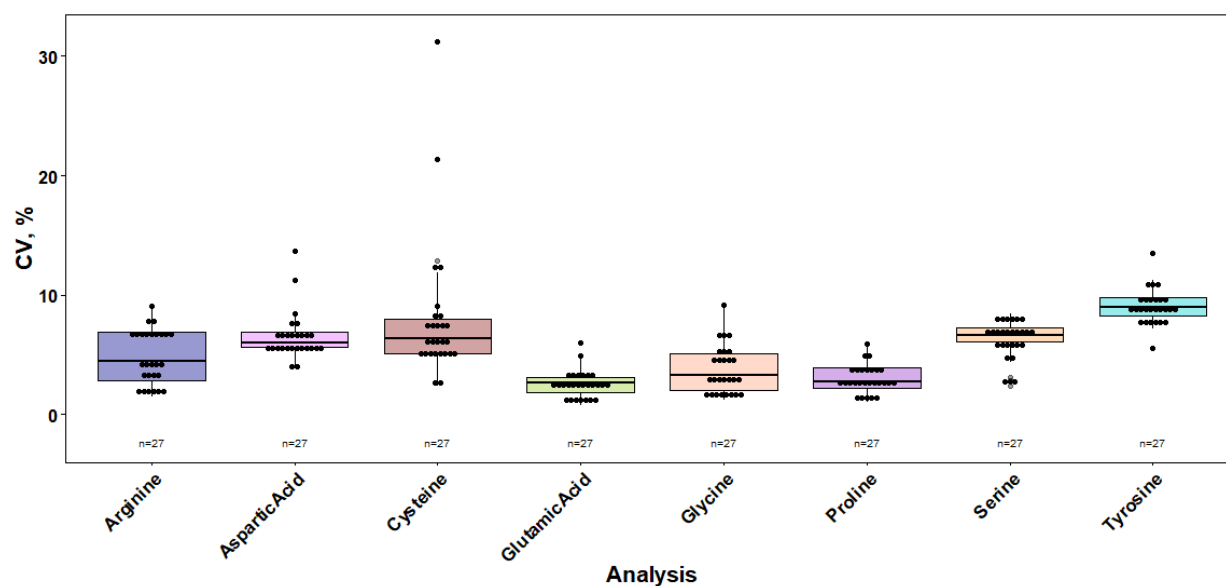
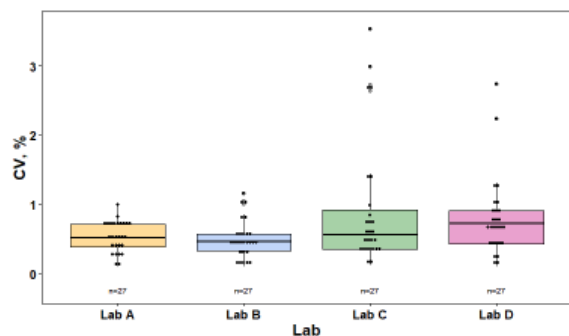


Figure 3.3 Between laboratory coefficient of variation (CV) of soybean meal non-essential amino acids by analysis. Individual boxplots are presented for each analysis where each data point represents the CV calculated for a single soybean meal sample across four laboratories. Between laboratory CV was calculated using the mean values from each laboratory for a given analyte, with the standard deviation representing variation between laboratories, reflecting analytical reproducibility.

A) Crude protein



B) Dry matter

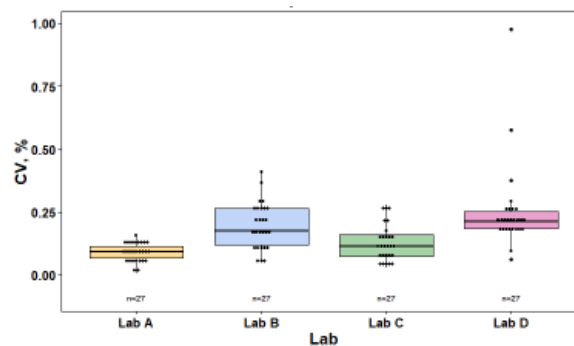


Figure 3.4 Within laboratory coefficient of variation (CV) of soybean meal A) crude protein and B) dry matter. Individual boxplots are presented for each laboratory where each data point represents the CV calculated for a single soybean meal sample analyzed in quadruplicate. Within laboratory CV was calculated using the mean and standard deviation of the four independent subsamples analyzed within each laboratory, representing analytical repeatability.

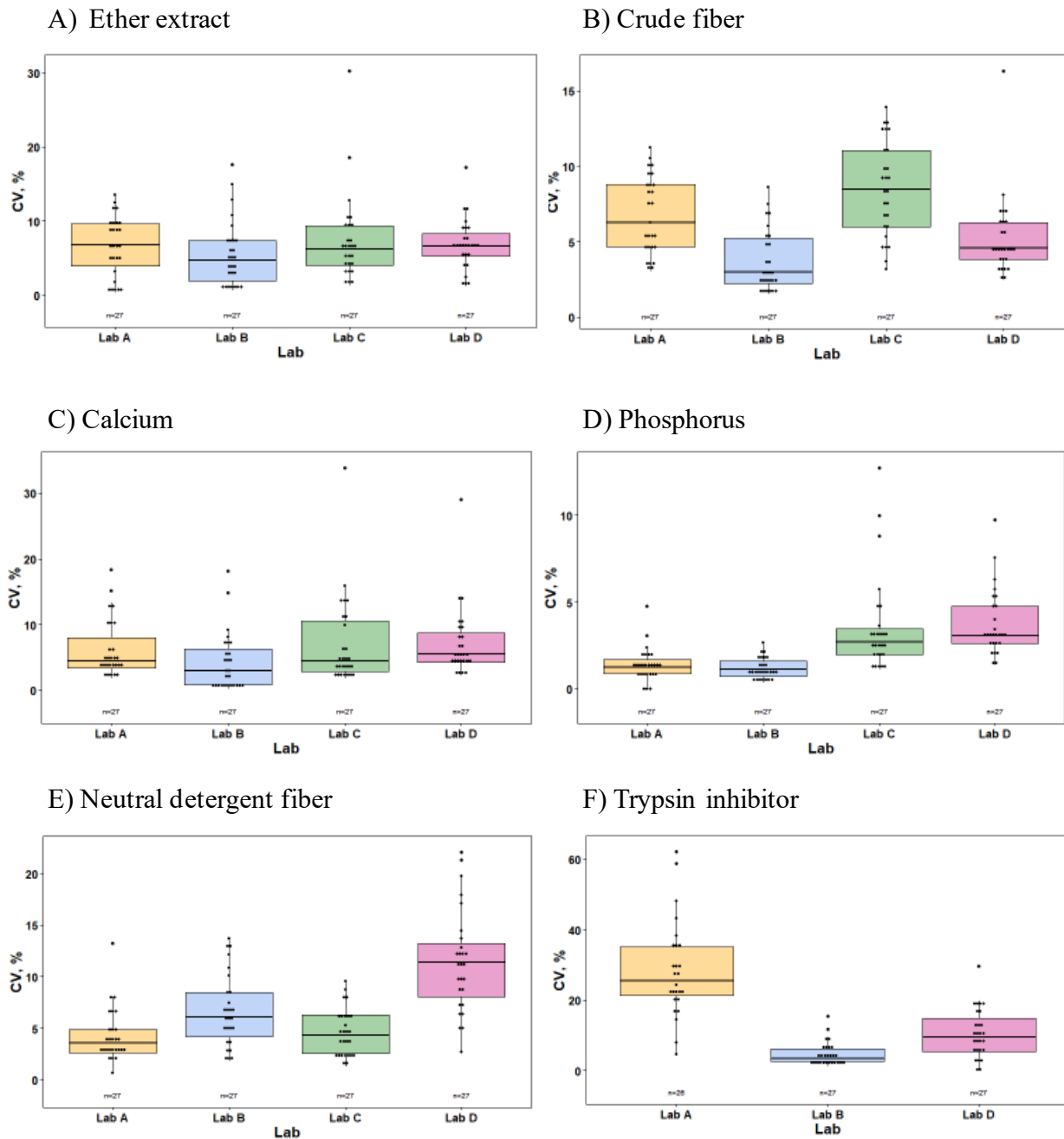


Figure 3.5 Within laboratory coefficient of variation (CV) of soybean meal A) ether extract, B) crude fiber, C) calcium, D) phosphorus, E) neutral detergent fiber, and F) trypsin inhibitor. Individual boxplots are presented for each laboratory where each data point represents the CV calculated for a single soybean meal sample analyzed in quadruplicate. Within laboratory CV was calculated using the mean and standard deviation of the four independent subsamples analyzed within each laboratory, representing analytical repeatability.

Table 3.1 Solvent extracted soybean meal composition means and ranges by laboratory (as-is basis).^{1,2}

Item, %	Lab A		Lab B		Lab C		Lab D	
	Mean ³	Range ⁴	Mean	Range	Mean	Range	Mean	Range
Dry matter	87.99 ± 0.44	86.74 - 88.85	87.93 ± 0.54	86.50 - 88.96	87.62 ± 0.53	86.04 - 88.37	88.61 ± 0.51	87.21 - 89.71
Crude protein	46.85 ± 0.76	45.26 - 48.46	46.78 ± 0.76	44.94 - 48.19	46.68 ± 0.87	43.60 - 48.30	46.54 ± 0.77	44.16 - 48.66
Ether extract	2.03 ± 0.70	0.91 - 4.71	1.72 ± 0.72	0.75 - 3.89	1.52 ± 0.75	0.48 - 3.93	2.13 ± 0.74	1.08 - 4.35
Crude fiber	3.46 ± 0.41	2.60 - 4.40	3.28 ± 0.39	2.40 - 4.40	3.11 ± 0.58	2.15 - 5.04	3.41 ± 0.41	2.51 - 4.67
Ash	6.27 ± 0.37	5.46 - 7.08	6.01 ± 0.30	5.15 - 6.80	6.73 ± 0.33	5.94 - 7.66	6.19 ± 0.34	5.66 - 7.03
Neutral detergent fiber	6.54 ± 0.75	5.20 - 8.80	7.02 ± 0.84	4.80 - 9.30	6.54 ± 0.77	5.00 - 8.60	8.38 ± 1.39	5.44 - 12.26
Calcium	0.41 ± 0.15	0.23 - 0.88	0.39 ± 0.12	0.23 - 0.64	0.41 ± 0.13	0.23 - 0.79	0.41 ± 0.16	0.22 - 0.83
Phosphorus	0.66 ± 0.03	0.57 - 0.71	0.66 ± 0.03	0.59 - 0.74	0.70 ± 0.04	0.57 - 0.84	0.65 ± 0.04	0.53 - 0.77

¹One sample from each soybean processing plant collected from June to August 2025 was ground and sent for analysis in quadruplicate at ATC Scientific (Little Rock, AR), Eurofins Food Chemistry Testing Center (Des Moines, IA), Midwest Laboratories (Omaha, NE), and University of Missouri (Columbia, MO).

²There were 108 samples analyzed by each laboratory, representing four replicate samples from each of the 27 soybean processing plants. A total of 100 samples were solvent extracted and 8 samples were extruded-expelled.

³Means represent the average value ± standard deviation for each individual assay within laboratory.

⁴The range is the minimum and maximum value for each individual assay within laboratory.

Table 3.2 Extruded-expelled soybean meal composition means and ranges by laboratory (as-is basis).^{1,2}

Item, %	Lab A		Lab B		Lab C		Lab D	
	Mean ³	Range ⁴	Mean	Range	Mean	Range	Mean	Range
Dry matter	91.92 ± 2.63	89.43 - 94.47	92.05 ± 2.50	89.60 - 94.56	91.78 ± 2.60	89.31 - 94.37	92.57 ± 2.61	89.92 - 95.06
Crude protein	41.34 ± 4.22	37.00 - 45.51	40.74 ± 4.37	36.13 - 44.94	40.46 ± 4.28	36.10 - 45.50	40.58 ± 4.51	36.19 - 46.22
Ether extract	9.48 ± 0.46	9.04 - 10.13	9.32 ± 0.50	8.76 - 9.86	9.44 ± 0.62	8.78 - 10.50	9.69 ± 0.49	9.15 - 10.28
Crude fiber	5.54 ± 0.56	4.90 - 6.70	5.26 ± 0.37	4.90 - 5.70	4.73 ± 0.41	4.10 - 5.31	5.44 ± 0.39	4.87 - 6.02
Ash	5.37 ± 0.47	4.84 - 6.07	5.25 ± 0.20	4.93 - 5.49	5.94 ± 0.30	5.52 - 6.31	5.26 ± 0.18	5.09 - 5.50
Neutral detergent fiber	9.59 ± 0.66	8.90 - 11.20	10.00 ± 0.71	8.90 - 11.10	9.01 ± 0.53	8.30 - 9.70	9.21 ± 0.65	8.30 - 10.49
Calcium	0.29 ± 0.01	0.27 - 0.30	0.28 ± 0.01	0.28 - 0.28	0.28 ± 0.01	0.27 - 0.30	0.29 ± 0.01	0.27 - 0.31
Phosphorus	0.56 ± 0.03	0.53 - 0.60	0.57 ± 0.03	0.54 - 0.61	0.61 ± 0.03	0.57 - 0.64	0.55 ± 0.03	0.51 - 0.60

¹One sample from each soybean processing plant collected from June to August 2025 was ground and sent for analysis in quadruplicate at ATC Scientific (Little Rock, AR), Eurofins Food Chemistry Testing Center (Des Moines, IA), Midwest Laboratories (Omaha, NE), and University of Missouri (Columbia, MO).

²There were 108 samples analyzed by each laboratory, representing four replicate samples from each of the 27 soybean processing plants. A total of 100 samples were solvent extracted and 8 samples were extruded-expelled.

³Means represent the average value ± standard deviation for each individual assay within laboratory.

⁴The range is the minimum and maximum value for each individual assay within laboratory.

Table 3.3 Solvent extracted soybean meal amino acid profile means and ranges by laboratory (as-is basis).^{1,2}

Amino acid, % ³	Lab A		Lab B		Lab C		Lab D	
	Mean ⁴	Range ⁵	Mean	Range	Mean	Range	Mean	Range
Essential								
Arginine	3.32 ± 0.15	2.85 - 3.61	3.32 ± 0.09	3.03 - 3.55	3.58 ± 0.34	2.34 - 4.46	3.39 ± 0.15	3.13 - 4.00
Histidine	1.20 ± 0.05	1.06 - 1.34	1.22 ± 0.02	1.11 - 1.29	1.20 ± 0.06	1.04 - 1.41	1.26 ± 0.05	1.19 - 1.43
Isoleucine	2.16 ± 0.08	1.81 - 2.31	2.19 ± 0.05	2.01 - 2.28	1.92 ± 0.17	1.57 - 2.28	2.29 ± 0.11	2.13 - 2.72
Leucine	3.53 ± 0.16	3.12 - 3.89	3.66 ± 0.07	3.36 - 3.79	3.36 ± 0.15	2.89 - 3.79	3.65 ± 0.15	3.42 - 4.27
Lysine	2.98 ± 0.12	2.50 - 3.19	2.94 ± 0.06	2.73 - 3.08	3.01 ± 0.21	2.59 - 4.01	3.07 ± 0.11	2.89 - 3.54
Methionine	0.60 ± 0.04	0.51 - 0.76	---	---	0.63 ± 0.03	0.52 - 0.70	0.65 ± 0.03	0.61 - 0.79
Phenylalanine	2.33 ± 0.08	2.10 - 2.49	2.39 ± 0.05	2.19 - 2.47	2.21 ± 0.08	1.98 - 2.36	2.46 ± 0.12	2.25 - 2.95
Threonine	1.77 ± 0.11	1.25 - 1.95	1.87 ± 0.05	1.74 - 1.97	1.68 ± 0.07	1.43 - 1.93	1.86 ± 0.10	1.74 - 2.29
Tryptophan	0.63 ± 0.06	0.51 - 0.77	---	---	0.63 ± 0.08	0.50 - 0.82	0.63 ± 0.03	0.54 - 0.71
Valine	2.21 ± 0.08	1.86 - 2.36	2.26 ± 0.05	2.09 - 2.37	2.05 ± 0.21	1.63 - 2.82	2.36 ± 0.09	2.16 - 2.71
Non-essential								
Alanine	2.04 ± 0.07	1.80 - 2.20	2.03 ± 0.04	1.88 - 2.12	2.02 ± 0.12	1.72 - 2.46	2.05 ± 0.08	1.95 - 2.36
Aspartic Acid	6.10 ± 0.37	5.06 - 6.97	5.38 ± 0.12	4.92 - 5.63	5.57 ± 0.28	4.86 - 6.20	5.35 ± 0.24	5.04 - 6.40
Cysteine	0.68 ± 0.15	0.35 - 1.55	---	---	---	---	0.72 ± 0.05	0.64 - 0.90
Glutamic Acid	8.89 ± 0.44	6.47 - 9.72	8.59 ± 0.18	7.81 - 8.95	8.74 ± 0.43	7.40 - 9.88	8.82 ± 0.44	8.25 - 10.90
Glycine	1.93 ± 0.08	1.65 - 2.13	1.99 ± 0.04	1.85 - 2.06	2.08 ± 0.14	1.75 - 2.46	1.99 ± 0.08	1.88 - 2.32
Hydroxylysine	< 0.050	< 0.050	---	---	---	---	0.06 ± 0.02	0.03 - 0.14
Hydroxyproline	0.25 ± 0.07	0.14 - 0.44	---	---	---	---	0.07 ± 0.02	0.00 - 0.18
Ornithine	0.12 ± 0.08	0.00 - 0.21	---	---	---	---	0.04 ± 0.01	0.03 - 0.08
Proline	2.32 ± 0.08	2.09 - 2.50	2.39 ± 0.07	2.21 - 2.57	2.39 ± 0.14	2.10 - 2.93	2.42 ± 0.15	1.71 - 2.83
Serine	2.36 ± 0.14	1.43 - 2.78	2.38 ± 0.05	2.16 - 2.49	2.29 ± 0.20	1.58 - 2.68	2.11 ± 0.14	1.94 - 2.86

Taurine	< 0.050	< 0.050	---	---	---	---	0.12 ± 0.02	0.07 - 0.20
Tyrosine	1.83 ± 0.15	1.39 - 2.15	1.49 ± 0.05	1.35 - 1.63	1.68 ± 0.06	1.47 - 1.84	1.69 ± 0.12	1.38 - 2.03

¹One sample from each soybean processing plant collected from June to August 2025 was ground and sent for analysis in quadruplicate at ATC Scientific (Little Rock, AR), Eurofins Food Chemistry Testing Center (Des Moines, IA), Midwest Laboratories (Omaha, NE), and University of Missouri (Columbia, MO).

²There were 108 samples analyzed by each laboratory, representing four replicate samples from each of the 27 soybean processing plants. A total of 100 samples were solvent extracted and 8 samples were extruded-expelled.

³Values not reported (---) indicate the analyte was not included in standard amino acid analysis package.

⁴Means represent the average value ± standard deviation for each individual assay within laboratory.

⁵The range is the minimum and maximum value for each individual assay within laboratory.

Table 3.4 Extruded-expelled soybean meal amino acid profile means and ranges by laboratory (as-is basis).^{1,2}

Amino acid, % ⁵	Lab A		Lab B		Lab C		Lab D	
	Mean ³	Range ⁴	Mean	Range	Mean	Range	Mean	Range
Essential								
Arginine	2.86 ± 0.32	2.44 - 3.24	2.87 ± 0.32	2.51 - 3.20	2.98 ± 0.36	2.62 - 3.49	2.96 ± 0.34	2.56 - 3.30
Histidine	1.06 ± 0.11	0.93 - 1.22	1.07 ± 0.10	0.97 - 1.17	1.05 ± 0.11	0.91 - 1.18	1.12 ± 0.12	0.98 - 1.26
Isoleucine	1.87 ± 0.19	1.65 - 2.09	1.90 ± 0.20	1.68 - 2.11	1.79 ± 0.19	1.43 - 2.00	2.01 ± 0.23	1.74 - 2.25
Leucine	3.08 ± 0.32	2.73 - 3.55	3.21 ± 0.29	2.91 - 3.50	3.01 ± 0.26	2.70 - 3.29	3.20 ± 0.31	2.83 - 3.52
Lysine	2.61 ± 0.28	2.26 - 2.94	2.59 ± 0.26	2.33 - 2.85	2.54 ± 0.27	2.26 - 2.93	2.71 ± 0.29	2.41 - 3.02
Methionine	0.52 ± 0.04	0.48 - 0.58	---	---	0.58 ± 0.05	0.52 - 0.65	0.58 ± 0.05	0.52 - 0.63
Phenylalanine	2.04 ± 0.23	1.79 - 2.35	2.08 ± 0.22	1.86 - 2.31	1.97 ± 0.22	1.67 - 2.19	2.15 ± 0.23	1.83 - 2.39
Threonine	1.54 ± 0.14	1.40 - 1.75	1.67 ± 0.15	1.51 - 1.82	1.42 ± 0.16	1.16 - 1.63	1.63 ± 0.16	1.45 - 1.83
Tryptophan	0.53 ± 0.09	0.42 - 0.69	---	---	0.45 ± 0.08	0.37 - 0.53	0.55 ± 0.06	0.47 - 0.63
Valine	1.92 ± 0.19	1.71 - 2.15	1.96 ± 0.20	1.75 - 2.18	1.86 ± 0.18	1.49 - 2.05	2.07 ± 0.24	1.78 - 2.32
Non-essential								
Alanine	1.81 ± 0.19	1.58 - 2.10	1.80 ± 0.17	1.62 - 1.98	1.74 ± 0.17	1.57 - 2.06	1.80 ± 0.17	1.62 - 1.99
Aspartic Acid	5.24 ± 0.58	4.40 - 5.93	4.72 ± 0.46	4.24 - 5.17	4.61 ± 0.48	4.11 - 5.37	4.65 ± 0.48	4.15 - 5.21
Cysteine	0.60 ± 0.04	0.56 - 0.66	---	---	---	---	0.66 ± 0.04	0.61 - 0.71
Glutamic Acid	7.61 ± 0.67	6.73 - 8.46	7.48 ± 0.70	6.77 - 8.20	7.31 ± 0.81	6.47 - 8.50	7.58 ± 0.78	6.75 - 8.43
Glycine	1.71 ± 0.18	1.50 - 1.99	1.79 ± 0.18	1.61 - 1.96	1.78 ± 0.18	1.60 - 2.06	1.79 ± 0.18	1.58 - 1.98
Hydroxylysine	< 0.050	< 0.050	---	---	---	---	0.07 ± 0.02	0.04 - 0.10
Hydroxyproline	0.26 ± 0.06	0.19 - 0.35	---	---	---	---	0.09 ± 0.02	0.07 - 0.12
Ornithine	0.05 ± 0.01	0.00 - 0.08	---	---	---	---	0.04 ± 0.02	0.02 - 0.07
Proline	2.06 ± 0.21	1.83 - 2.35	2.16 ± 0.21	1.91 - 2.38	2.02 ± 0.18	1.86 - 2.41	2.16 ± 0.23	1.84 - 2.48
Serine	2.03 ± 0.18	1.82 - 2.28	2.08 ± 0.21	1.84 - 2.29	1.80 ± 0.35	1.24 - 2.43	1.84 ± 0.18	1.64 - 2.08

Taurine	< 0.050	< 0.050	---	---	---	---	0.15 ± 0.03	0.11 - 0.19
Tyrosine	1.63 ± 0.18	1.46 - 1.95	1.23 ± 0.16	1.05 - 1.44	1.46 ± 0.15	1.28 - 1.64	1.50 ± 0.16	1.21 - 1.71

¹One sample from each soybean processing plant collected from June to August 2025 was ground and sent for analysis in quadruplicate at ATC Scientific (Little Rock, AR), Eurofins Food Chemistry Testing Center (Des Moines, IA), Midwest Laboratories (Omaha, NE), and University of Missouri (Columbia, MO).

²There were 108 samples analyzed by each laboratory, representing four replicate samples from each of the 27 soybean processing plants. A total of 100 samples were solvent extracted and 8 samples were extruded-expelled.

³Values not reported (---) indicate the analyte was not included in standard amino acid analysis package.

⁴Means represent the average value ± standard deviation for each individual assay within laboratory.

⁵The range is the minimum and maximum value for each individual assay within laboratory.

Table 3.5 Soybean meal trypsin inhibitor means and ranges by soybean meal type and laboratory (as-is basis).^{1,2}

Type	Lab A, TIA mg/g		Lab B, TIU/mg		Lab D, TIU/mg	
	Mean ³	Range ⁴	Mean	Range	Mean	Range
Samples, n	108		108		81	
Solvent extracted	2.18 ± 0.86	0.55 - 4.39	5.61 ± 1.13	2.37 - 7.85	4.94 ± 0.45	1.98 - 9.43
Extruded-expelled	3.59 ± 1.23	1.18 - 5.12	13.83 ± 1.50	11.70 - 16.10	13.88 ± 2.50	12.66 - 15.09

¹One sample from each soybean processing plant collected from June to August 2025 was ground and sent for analysis in quadruplicate at ATC Scientific (Little Rock, AR) and Eurofins Food Chemistry Testing Center (Des Moines, IA) and was analyzed in triplicate at USDA-ARS (Columbia, MO).

²A representative sample was collected from 27 different US soybean processing plants between June and August 2025. Of the 27 plants, 25 were solvent extracted and 2 plants were extruded-expelled mechanically.

³Means represent the average value ± standard deviation for each individual assay within laboratory.

⁴The range is the minimum and maximum value for each individual assay within laboratory.