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Assessment of Sampling Technique from Feeders on Copper, Zinc, Calcium, and Phosphorus Analysis

A.M. Jones, J.C. Woodworth, C.I. Vahl,[1] S.S. Dritz,[2] M.D. Tokach, R.D. Goodband, and J.M. DeRouchey

Summary

While numerous research articles have been published on how to collect a representative sample, and analytical or laboratory-to-laboratory variation, we are unaware of any studies to examine exactly how many samples to collect from feeders, or whether they should be pooled or not to minimize analytical variation. Therefore, this study was designed to evaluate different sampling procedures and the number of samples to collect, and achieve an accurate assessment of nutrient fortification in swine diets.

Diet samples were collected from a study evaluating the effects of increasing Cu on growth performance of finishing pigs. Treatments were arranged in a split-plot design with the whole-plot consisting of 1 of 6 concentrations of dietary Cu (27 to 147 ppm total Cu included in the diet) and the subplot using 1 of 2 sampling techniques (probe vs. hand grab). In addition to Cu, samples were also analyzed for Ca, P, and Zn, which were formulated to be the same across diets. A total of 6 feeders per dietary treatment were sampled using a 63 in. brass open handle probe (Seedburo Equipment Company, Des Plaines, IL), which contained 10 openings spaced approximately 2 in. apart. The probe was inserted into the feeder on average 4 times to obtain ~2 lb of sample. Alternatively, samples were simply collected by inserting a bare hand into the feeder approximately 8 times to obtain the ~ 2 lb of sample. Within a feeder and sampling technique, subsamples (~200 g) were created by using a sample splitting device. Next, all samples were ground through a centrifugal mill (0.5 mm screen) and submitted for mineral analysis in duplicate. In addition to the 6 individual feeder samples, a subsample (~33 g) from each individual feeder was pooled within dietary treatment and sampling technique to form a single composite sample (~200 g). This process was repeated until 4 individual composite samples were created for each diet and sampling technique.

Results indicated that the observed variability when sampling feeders with an open handle probe was reduced ($P = 0.013$) for Cu and marginally reduced ($P = 0.058$) for Ca, when compared with hand-sampling. However, no evidence for differences was detected among sampling techniques for Zn and P for the individual feeder analysis.

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There was no evidence for differences detected among sampling techniques for Cu, Zn, Ca, and P when samples were pooled from 6 feeders to form a single composite sample. While not statistically significant, the overall variability was numerically reduced when pooled from 6 feeders to form a single composite sample. From these results, sampling frequency calculations were determined to assess sampling accuracy within a 95% confidence interval. Results indicated that the number of feeders or composite samples required to analyze was less regardless of Cu, Zn, Ca, and P when using a probe compared to a hand. In summary, these results would suggest that in general, sampling with a probe is associated with less variability on an individual sample basis, but when individual samples are pooled to form a composite sample, there was no difference among sampling techniques. Our results suggest that samples collected with a probe and composited would be the best option to minimize variation and analytical costs.

Introduction

The implementation and monitoring of quality control, quality assurance systems, and their standard operating procedures in feed mill operations are integral in assessing the overall success and profitability of livestock operations.[3] The proper sampling of finished feed and its subsequent analysis is a common standard operating procedure that is used for most swine nutrition studies to ensure that adequate diet manufacturing and delivery have been met; therefore, this analysis is serving as a control measure for both nutritionists and feed mill managers. While numerous research articles and bulletins have been published on how to collect a representative sample, as well as others describing analytical or laboratory-to-laboratory variation, we are unaware of any studies to examine exactly how many samples to collect from the feeders, or if they should be pooled or not to minimize analytical variation. Therefore, this study was designed to evaluate different sampling procedures and number of samples to collect from feeders within a swine facility to achieve an accurate assessment of nutrient fortification in swine diets.

Procedures

For this study, feed was manufactured at a commercial feed mill in southwestern Minnesota. Ingredients were added to a ribbon mixer (Scott Model 6013, New Prague, MN) in 6,000-lb batches and mixed for 60 sec. These mash diets were then transported and delivered to a commercial grow-finish swine barn. The barn contained 42 pens that were each equipped with 1 cup waterers and a 4-hole stainless-steel, dry self-feeders (36 in. tall × 60 in. long; Thorp Equipment, Thorp, WI) with approximately 300 lb of feed capacity. Feed additions to each individual pen were made and recorded by a robotic feeding system (FeedPro; Feedlogic Corp., Wilmar, MN).

A total of 36 feeders were used with 6 feeders per dietary treatment. This study was carried out as a split-plot design with the whole plot using 1 of 6 dietary Cu concentrations ranging from 27 to 147 ppm total Cu included in the diet, and the subplot using 1 of 2 sampling techniques from each feeder (probe vs. hand grab). The 6 dietary treatments (Table 1) consisted of: 3 corn-soybean meal-based diets with 20% corn dried distillers grains with solubles (DDGS) formulated to contain 0.91% SID Lys, and 33, 87, or 147 ppm of total Cu. The second set of corn-soybean meal-based diets contained 10% corn DDGS and was formulated to contain 0.65% SID Lys and 27, 81, or 141

[3] Richardson, C. R. 1996. Quality control in feed productions. Proc. Mid-South Nutr. Conf. p. 1-5.

ppm of total Cu, respectively. Copper sulfate (Prince Agri Products Inc., Quincy, IL) was added at 17, 70, and 130 ppm in diets A and D, B and E, and C and F, respectively. The remaining Cu making up the total Cu concentrations was provided by the corn, soybean meal, and corn DDGS. Nutrient profiles of the ingredients used in this study were based on NRC[4] values.

Two sampling techniques (hand vs. probe) were tested on a total of 6 feeders per dietary treatment. The first sampling technique utilized was randomized within feeder. A 63 in. brass open handle probe (Seedburo Equipment Company, Des Plaines, IL), which contained 10 openings spaced approximately 2 in. apart was used. The probe was inserted at a 45° angle in relation to the bottom of the feeder, with slots facing upward and closed. After the probe was fully inserted, the slots were opened and the probe was moved up and down (approximately 6 in.) in several short motions. The slots were then closed and the probe was removed from the feeder. Each sample obtained with a probe was approximately 250 g. Samples taken by hand were collected by inserting one's arm into the feeder at a depth of approximately 12 in. Next, the individual's hand, wrist, and forearm were rotated so that their palm was facing upward toward the top of the feeder with their fingers placed together and slightly bent. The individual then lifted their arm out of the feeder. Each sample collected by hand was approximately 125 g. Each sampling technique was repeated within a feeder until approximately 2 lb of total sample was collected; approximately 4 times with the probe and 8 times by hand. To prevent cross contamination, the probe and individual's arm were wiped clean between feeders with a towel (Scott Shop Towel, Kimberly-Clark Worldwide, Inc., Dallas, TX). All samples were collected by the same individual. Samples were then transported back to the Kansas State University Swine Nutrition Lab (Manhattan, KS) where they were stored at -4°F.

Samples were split using a riffle splitter (Humboldt Mfg. Co., Norridge, IL) and ground using a 0.5 mm screen (Retsch Ultra Centrifugal Mill ZM 200; Haan, Germany) prior to compositing and analysis. A 200 g subsample from each individual feeder and sampling technique was collected for analysis. In addition, a subsample (~33 g) from each individual feeder and sampling technique was collected and pooled within dietary treatment and sampling technique to form a 200 g composite sample. This process was repeated until 4 individual composite samples were created for each diet and sampling technique. All samples were submitted to Cumberland Valley Analytical Services (Hagerstown, MD) and analyzed for Cu, Zn, Ca, and P.

Data were analyzed as a complete randomized design using the PROC MIXED procedure in SAS version 9.4 (SAS Institute, Inc., Cary, NC) using its default estimation method REML and Kenward-Rodger's procedure used to estimate degrees of freedom and adjust the estimated SE for bias correction.[5] Diet or composite sample served as the experimental unit for the whole plot and the individual sample serving as the experimental unit for the subplot. For the individual feeder analysis, diet and sampling technique, and diet × sampling technique interaction served as the fixed effects with diet nested within feeder. The interactions of feeder × diet nested within sampling

[4] NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.
[5] Littell, R. C., G. A. Milliken, W. W. Stroup, R. D. Wolfinger, and O. Schabenberger. 2006. SAS® for mixed models, 2nd ed. SAS Inst. Inc., Cary, NC.

technique were included as the random effects. The following base statistical model was used in the analysis: $Y_{ijkl} = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + e_{ijkl,}$ in which Y is the response criterion, μ is the overall mean, τ_i is the effect of the ith diet (i = 1, 2, 3, ..., 6), β_j is the jth effect of sampling technique (j = 1, 2), $(\tau\beta_{ij})$ is the interaction effect between the ith diet and jth sampling technique, and e_{ijkl} is the error term with lth duplicate (l = 1, 2). The model for the composite sample analysis included the composite, diet, and sampling technique, and composite × sampling technique interaction. Random effects included diet nested within composite and the interaction of composite × diet nested within sampling technique. Means were analyzed using the LSMEANS statement of SAS, with least squares means calculated for each independent variable.

To assess the variability between sampling techniques, we expanded our models to accommodate heterogeneous residual variances: $e_{ijk} \sim$ iid N $(0, \sigma^2)$. Next, we tested whether or not the numerical differences between the variances for sampling technique were significant; a likelihood ratio test was used comparing the goodness of the fit of the homogenous residual variances of the base model (referred to as the reduced model) and heterogenous residual variances (full model).[6] Next, chi square analysis was used to evaluate the difference between the restricted log likelihood of the reduced model and restricted log likelihood of the full model using a chi square (x^2) distribution with 1 degree of freedom. Results were considered significant at $P \leq 0.05$ and marginally significant between $P > 0.05$ and $P \leq 0.10$.

Next, residual variance estimates from the reduced model were used in sampling frequency calculations to determine sampling accuracy within a 95% confidence interval. To assess this, a margin of analysis was utilized (Equation 1). Where n is the number of samples, $Z^2_{a/2}$ is the critical value for a normal distribution given a desired confidence level (95%), σ^2 is the variance estimate of the population for a given sample, e^2 is the margin of error no larger than a given concentration (i.e. ppm). It is important to note, that the variance for the residual from the covariance estimate was divided by 2 since each sample was analyzed in duplicate at the lab. We then calculated the margin of error using the observed variances for the hand and probe samples for the individual and composite feeder analysis.

$$n \approx \frac{z^2_{a/2}(\sigma^2)}{e^2} \quad \text{(Equation 1)}$$

Results and Discussion

To determine whether the magnitude of differences between sampling techniques were significant, we used a chi-square analyses to evaluate the likelihood ratios comparing models accounting for heterogenous variance vs. those that assumed homogenous variance. The observed variability (Table 2) when sampling feeders with an open handle probe was significantly reduced ($P = 0.013$) for Cu (Figures 1 to 4) and marginally reduced ($P = 0.058$) for Ca (Figures 9 to 12) on the individual feeder analysis, which can be seen in Figures 1, 2, 9, and 10. There was no evidence for differences detected

[6] Stroup, W. W. 2013. Inference, part II: covariance components. In: F. Dominici, J. J. Faraway, M. Tanner, and J. Zidek, editors. Generalized Linear Mixed Models. CRC Press Taylor and Francis Group, Boca Raton, FL. p. 179 – 199.

between sampling technique for Zn (Figures 5 to 8) and P (Figures 12 to 16) for the individual feeder analysis. Interestingly, when samples were pooled within sampling technique and dietary treatment to form a composite, there was no evidence for differences detected between sampling techniques for Cu, Zn, Ca, and P. Thus, these results would suggest that pooling samples to form a homogenized composite sample reduced total variability. Intuitively, this would be expected due to a homogenized sample being theoretically equal in composition throughout.

From these results, sampling frequency calculations were determined to assess sampling accuracy within a 95% confidence interval. To facilitate this, a margin of error analysis was utilized to estimate the mean concentration of a given diet with n samples and a margin of error (±) from the expected mean (Equation 1 in procedures). Covariance parameter estimates generated from the heterogenous variances (full model) for Cu, Zn, Ca, and P were utilized in the calculation. Examples using the sampling frequency calculations are reported in Tables 5 and 6. For instance, if we wanted to estimate the mean concentration of 100 ppm Cu with a margin of error no larger or smaller than 15 ppm of Cu using a 95% confidence interval, we would need to sample 17 feeders by hand and 7 feeders by probe when analyzing Cu on an individual feeder analysis. Based on our pooling of samples from 6 feeders we would need to submit 4 composite samples if sampling by hand and 2 composite samples if collected with a probe. Based on these results, it is clear that feed samples collected with probe require fewer feeders to be sampled. This seems to indicate that a probed and pooled sample will lead to a lower number of samples and thus lower analytic cost for a given margin of error. One caution with the composite analysis is that this applies to composites of 6 feeders. Further investigation is needed to determine the optimum number of feeders that would be needed to make the composite pools.

In conclusion, equations can be used to generate the sample size needed to accurately determine nutrient concentrations in a diet. Our results suggest that the best option to minimize variation and reduce analytical cost is to collect samples with a probe from 6 feeders, and composite before analysis.

Table 1. Diet composition, (as-fed basis)

	Diets[1]	
Item, %	A, B, and C	D, E, and F
Corn	61.33	79.48
Soybean meal, 46.0% CP	16.52	8.39
Corn DDGS[2]	20.00	10.00
Calcium carbonate	1.20	1.13
Monocalcium P, 21.5% P	---	0.09
Salt	0.35	0.35
L-Lys HCl	0.37	0.32
L-Thr	0.04	0.07
L-Trp	0.01	0.02
Phytase[3]	0.01	0.01
Trace mineral premix[4]	0.10	0.10
Vitamin premix[5]	0.08	0.05
Copper sulfate[6]	---	---
Total	100	100
Calculated analysis		
Standardized ileal digestible (SID) amino acids, %		
Lys	0.91	0.65
Ile:Lys	62	59
Leu:Lys	159	166
Met:Lys	29	30
Met and Cys:Lys	56	59
Thr:Lys	61	65
Trp:Lys	18.5	18.5
Val:Lys	70	70
ME, kcal/lb	1,508	1,511
NE, kcal/lb	1,119	1,038
CP, %	18.1	12.9
Ca, %	0.55	0.50
P, %	0.40	0.34
Available P, %	0.26	0.22

[1] Diets A, B, and C were formulated for pigs ranging from 50 to 75 kg, while diets D, E, and F were for pigs ranging from 100 to 130 kg.

[2] Corn distillers dried grains with solubles.

[3] Optiphos 2000 (Huvepharma, Sofia, Bulgaria) provided 626 phytase units (FTU/kg) of diet with a release of 0.11% available P.

[4] Provided per kg of premix: zinc 11,000 mg; iron 11,000 mg; manganese 3,000 mg; copper 1,700 mg; iodine 330 mg; and selenium 300 mg.

[5] Provided per kg of premix: vitamin A 7,054,720 IU; vitamin D_3 1,102,300 IU; vitamin E 35,274 IU; vitamin B_{12} 26 mg; riboflavin (B2) 6,173 mg; niacin 39,683 mg; d-pantothenic acid 22,046 mg; and menadione 3,527 mg per kg.

[6] $CuSO_4$, copper sulfate (Prince Agri Products Inc., Quincy, IL) was added at 17, 70, and 130 ppm in diets A and D, B and E, and C and F, respectively.

Table 2. Evaluation for difference in variance between hand and probe sampling technique on Cu, Zn, Ca, and P using a chi-square test based on the likelihood ratio[1]

Analysis[2]	Parameters[3]	Chi square statistic[4]	Probability, $P <$
Cu			
Individual feeder	Hand vs. probe	6.2	0.013
Composite sample	Hand vs. probe	0.3	0.584
Zn			
Individual feeder	Hand vs. probe	0.1	0.752
Composite sample	Hand vs. probe	0.0	1.000
Ca			
Individual feeder	Hand vs. probe	3.6	0.058
Composite sample	Hand vs. probe	1.1	0.294
P			
Individual feeder	Hand vs. probe	0.4	0.527
Composite sample	Hand vs. probe	1.5	0.221

[1] The likelihood ratio test for covariance parameter estimates is a statistical test used to compare the goodness of fit of the heterogenous variance model allowing us to partition out the variances attributed to each sampling technique (hand and probe) to the homeogenous variance model that assumes the variances are the same.

[2] Mineral analysis on an individual feeder basis refers to the chi-square test based on the likelihood ratio from 6 individual feeders per dietary treatment and sampling technique. Whereas, the analysis on a composite feeder basis refers to the chi-square test based on the likelihood ratio when a subsample from each individual feeder was pooled within dietary treatment and sampling technique to form a single composite sample with a total of 4 composite samples created.

[3] Hand vs. probe: samples were collected by inserting one's hand into a feeder or using inserting an open handle brass probe into feeders.

[4] Chi square statistic was calculated by taking the difference between the restricted log likelihood of the heterogenous variance model and restricted log likelihood of homogenous variance model.

Table 3. Chemical analysis of dietary treatments for Cu, Zn, Ca, and P[1,2]

	Dietary treatment					
Item	A	B	C	D	E	F
Individual analysis[3]						
Cu, ppm	53 (33)	124 (87)	155 (147)	51 (27)	96 (81)	150 (141)
Zn, ppm	159 (127)	154 (127)	162 (127)	139 (121)	165 (121)	141 (121)
Ca, %	0.83 (0.55)	0.98 (0.55)	0.91 (0.55)	0.73 (0.50)	0.67 (0.50)	0.64 (0.50)
P, %	0.51 (0.40)	0.51 (0.40)	0.50 (0.40)	0.39 (0.40)	0.39 (0.40)	0.40 (0.40)
Composite analysis[4]						
Cu, ppm	55 (33)	110 (87)	163 (147)	66 (27)	88 (81)	151 (141)
Zn, ppm	151 (127)	154 (127)	145 (127)	153 (121)	145 (121)	139 (121)
Ca, %	0.78 (0.55)	0.83 (0.55)	0.92 (0.55)	0.58 (0.50)	0.63 (0.50)	0.56 (0.50)
P, %	0.51 (0.40)	0. 50 (0.40)	0.42 (0.40)	0.41 (0.40)	0.41 (0.40)	0.41 (0.40)

[1] All dietary samples were submitted to Cumberland Valley Analytical Services (Hagerstown, MD) for analysis. Values reported are the means for each mineral for both hand and probe samples.

[2] Values in parenthesis indicate formulated values.

[3] Complete diet samples were collected from 6 feeders and placed into a 1 gallon sampling bag that was labeled with pen number, diet, and sampling technique.

[4] A subsample from each individual feeder and sampling technique was collected and pooled with dietary treatment and sampling technique to form a composite sample. This process was repeated until 4 individual composite samples were created for each diet and sampling technique.

Table 4. Samples size calculations for a given margin of error and a 95% confidence interval[1]

	Cu				Zn			
	Individual feeder analysis[2]		Composite feeder analysis[3]		Individual feeder analysis[2]		Composite feeder analysis[3]	
	Hand[4]	Probe[5]	Hand[4]	Probe[5]	Hand[4]	Probe[5]	Hand[4]	Probe[5]
Margin of error, ppm	Number of feeders[6]		Number of samples[7]		Number of feeders[6]		Number of samples[7]	
± 2	967	375	220	140	306	268	140	135
± 4	242	94	55	35	77	67	35	34
± 6	107	42	24	16	34	30	16	15
± 8	60	23	14	9	19	17	9	8
± 10	39	15	9	6	12	11	6	5
± 15	17	7	4	2	5	5	2	2
± 20	10	4	2	1	3	3	1	1
± 25	6	2	1	1	2	2	1	1
± 30	4	2	1	1	1	1	1	1

[1] Values are calculated on the covariance parameter estimates obtained from the likelihood ratio test from the sampling and analysis of 6 feeders per dietary treatment.

[2] Individual feeder analysis: samples analyzed on an individual feeder basis.

[3] Composite feeder analysis: samples analyzed on 4 composite samples.

[4] Hand: samples taken by inserting one's hand into a feeder.

[5] Probe: samples taken with a 63 in. open handle brass probe into a feeder.

[6] Number of feeders: refers to the number of feeders that would need to be sampled to be within (±) a given margin of error on an individual feeder analysis basis.

[7] Number of samples: refers to the number of composite samples needed when pooling samples across 6 feeders to be within a given margin of error.

Table 5. Samples size calculations for a given margin of error and a 95% confidence interval[1]

	Ca				P			
	Individual feeder analysis[2]		Composite feeder analysis[3]		Individual feeder analysis[2]		Composite feeder analysis[3]	
	Hand[4]	Probe[5]	Hand[4]	Probe[5]	Hand[4]	Probe[5]	Hand[4]	Probe[5]
Margin of error, %	Number of feeders[6]		Number of samples[7]		Number of feeders[6]		Number of samples[7]	
± 2	169	84	87	53	4	5	4	2
± 4	42	21	22	13	1	1	1	1
± 6	19	9	10	6	1	1	1	1
± 8	11	5	5	3	1	1	1	1
± 10	7	3	3	2	1	1	1	1
± 15	5	2	2	1	1	1	1	1
± 20	3	2	2	1	1	1	1	1
± 25	3	1	1	1	1	1	1	1
± 30	2	1	1	1	1	1	1	1

[1] Values are calculated on the covariance parameter estimates obtained from the likelihood ratio test from the sampling and analysis of 6 feeders per dietary treatment and the pooling of the 6 feeders to form 4 composite samples.

[2] Individual feeder analysis: samples analyzed on an individual feeder basis.

[3] Composite feeder analysis: samples analyzed on 4 composite samples.

[4] Hand: samples taken by inserting one's hand into a feeder.

[5] Probe: samples taken with a 63 in. open handle brass probe into a feeder.

[6] Number of feeders: refers to the number of feeders that would need to be sampled to be within (±) a given margin of error on an individual feeder analysis basis.

[7] Number of samples: refers to the number of composite samples needed when pooling samples across 6 feeders to be within a given margin of error.

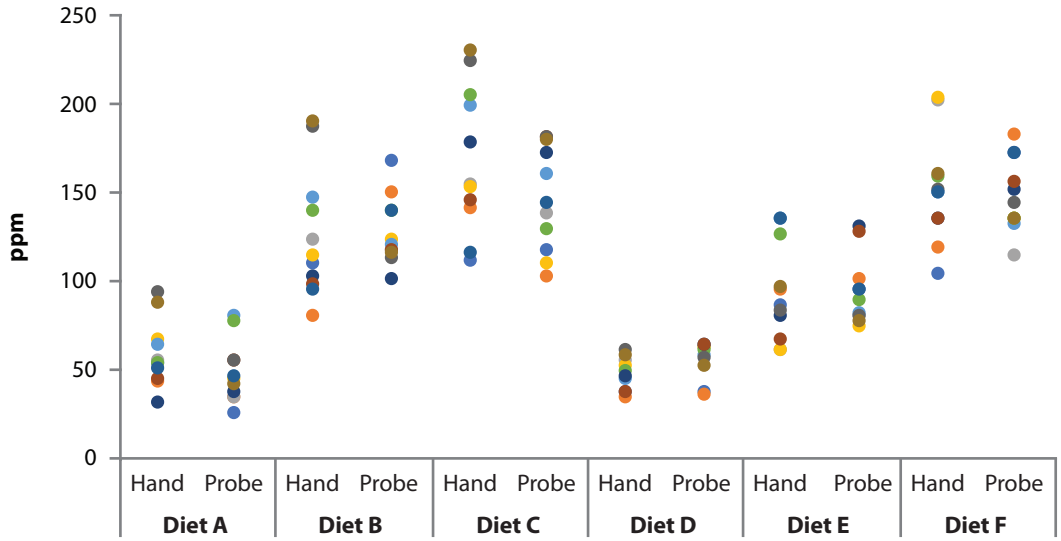

Figure 1. Distribution of analyzed mean Cu concentrations on an individual feeder basis. Each data point represents a single analysis of the 6 feeders in addition to its duplicate analysis for a total of 12 data observations for each sampling technique (hand: samples obtained by hand; probe: samples obtained using a 63 in. open handle probe) within a given dietary treatment. Diets A, B, and C contained 33, 87, and 147 ppm of total Cu; whereas, diets D, E, and F contained 27, 81, and 141 ppm of total Cu.

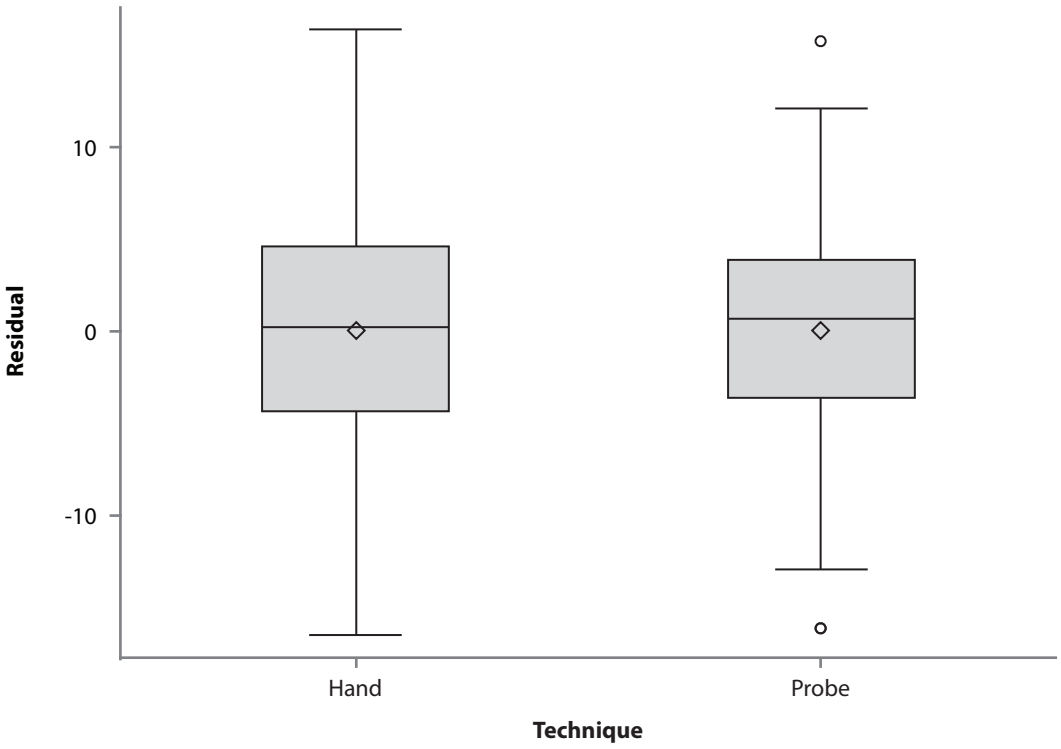

Figure 2. Statistical comparison examining the amount of variability attributed to samples obtained by hand and samples collected using a probe for Cu on an individual feeder basis.

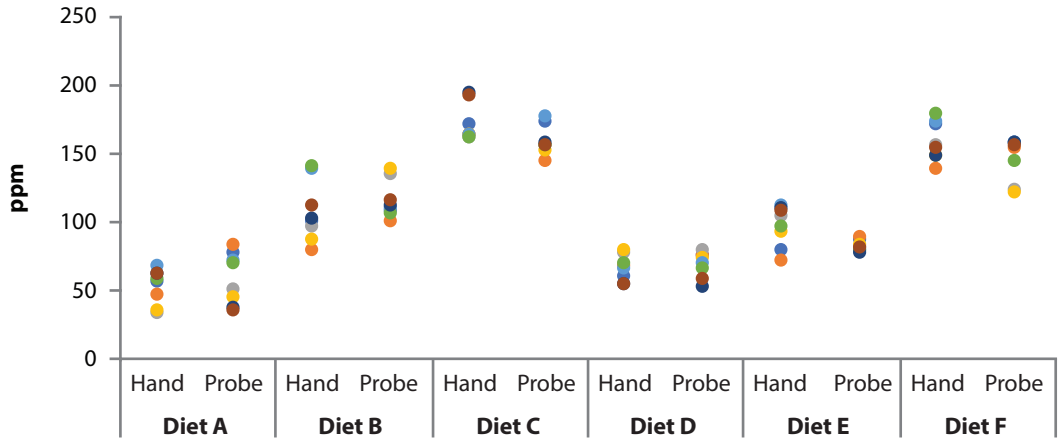

Figure 3. Distribution of analyzed mean Cu concentrations on a composite analysis basis in which subsamples from each of the 6 individual feeders were pooled within dietary treatment and sampling technique to form a single composite sample. The process was repeated until 4 individual composite samples were created for each diet and sampling technique. Each data point represents a single analysis on a composite sample in addition to its duplicate analysis for a total of 8 observations for each sampling technique (hand: samples obtained by hand; probe: samples obtained using a 63 in. open handle probe) within a given dietary treatment. Diets A, B, and C contained 33, 87, and 147 ppm of total Cu, whereas, diets D, E, and F contained 27, 81, and 141 ppm of total Cu.

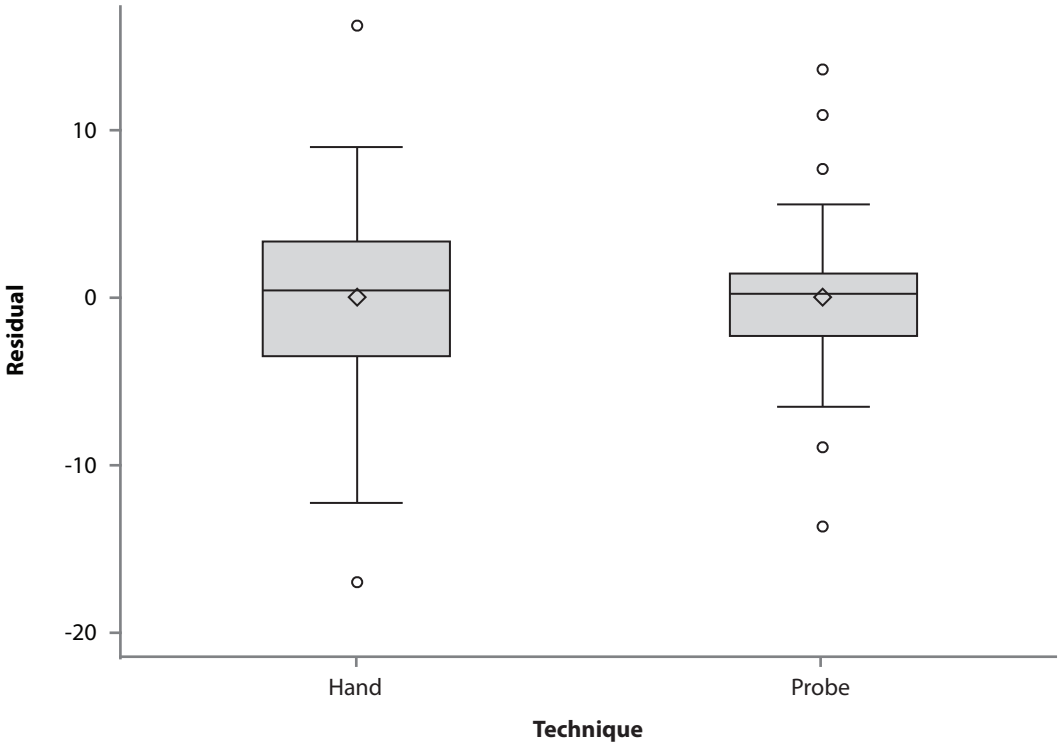

Figure 4. Statistical comparison examining the amount of variability attributed to samples obtained by hand and samples collected using a probe for the composite analysis of Cu.

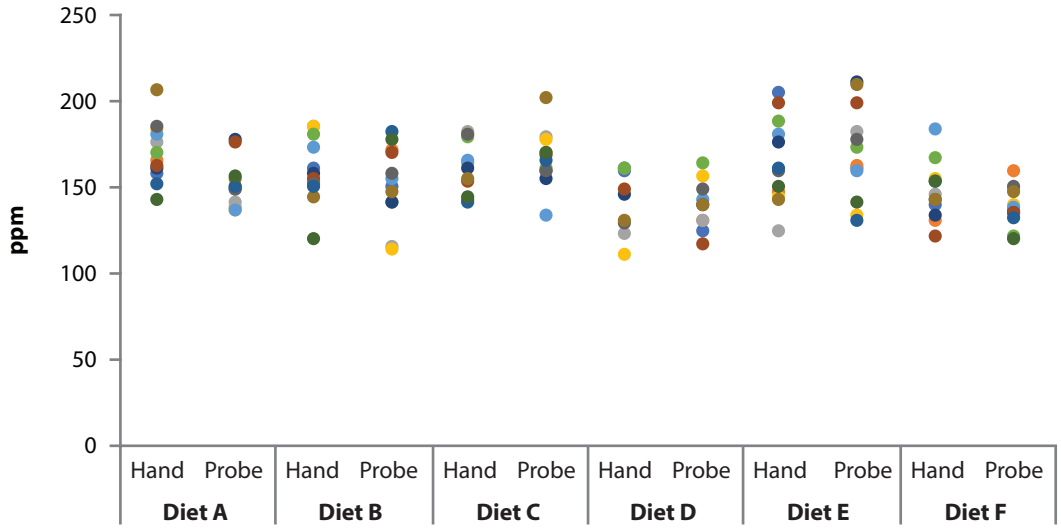

Figure 5. Distribution of analyzed mean Zn concentrations on an individual feeder basis. Each data point represents a single analysis of the 6 feeders in addition to its duplicate analysis for a total of 12 data observations for each sampling technique (hand: samples obtained by hand; probe: samples obtained using a 63 in. open handle probe) within a given dietary treatment. Diets A, B, and C contained 127 ppm Zn; whereas, diets D, E, and F contained 121 ppm Zn.

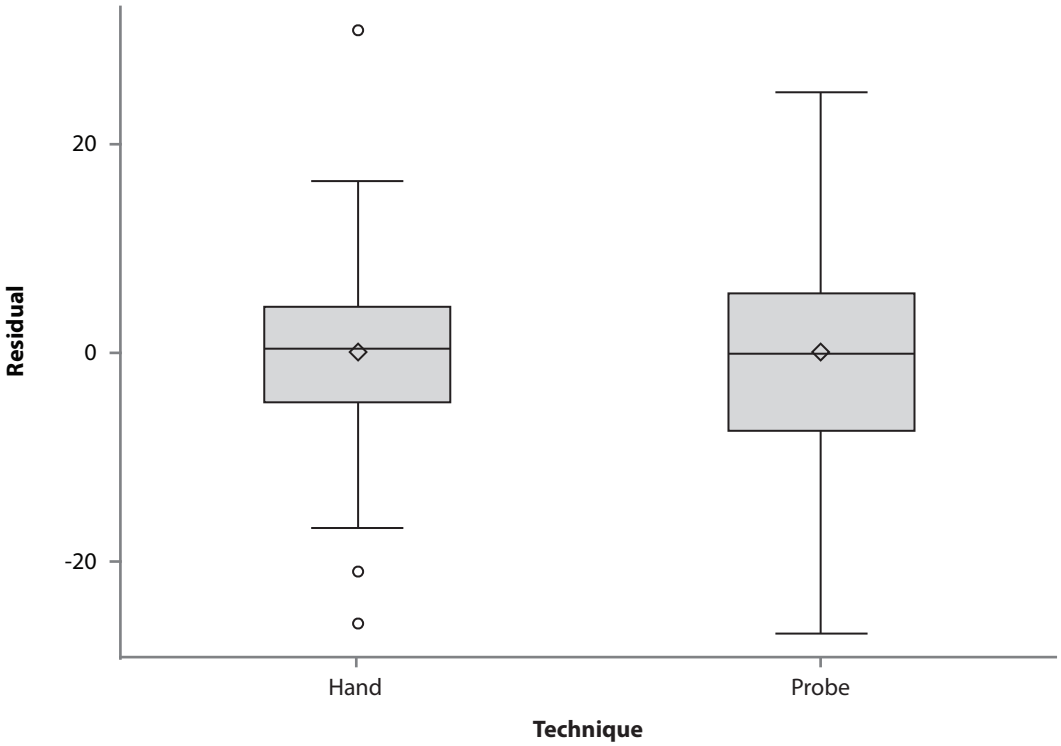

Figure 6. Statistical comparison examining the amount of variability attributed to samples obtained by hand and samples collected using a probe for Zn on an individual feeder basis.

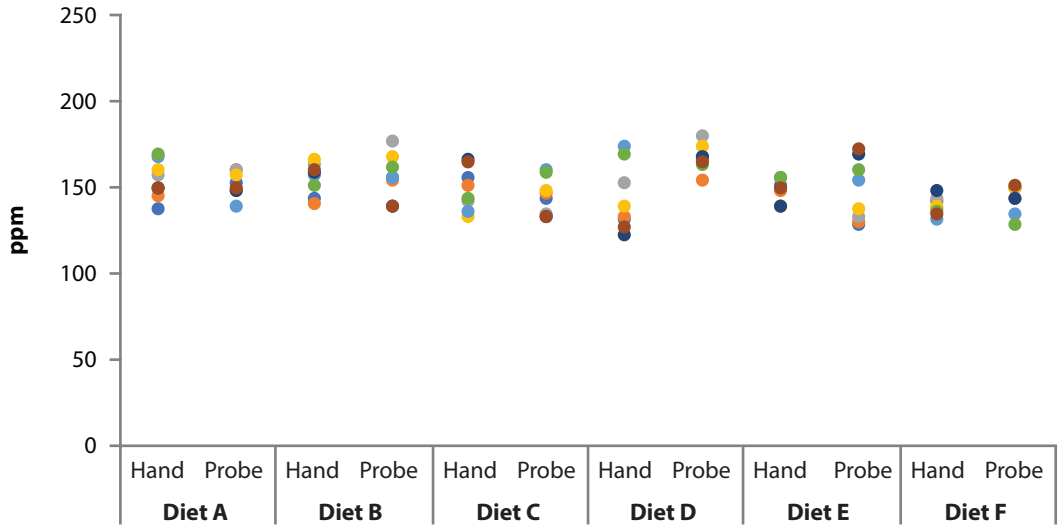

Figure 7. Distribution of analyzed mean Zn concentrations on a composite analysis basis in which subsamples from each of the 6 individual feeders were pooled within dietary treatment and sampling technique to form a single composite sample. The process was repeated until 4 individual composite samples were created for each diet and sampling technique. Each data point represents a single analysis on a composite sample in addition to its duplicate analysis for a total of 8 observations for each sampling technique (hand: samples obtained by hand; probe: samples obtained using a 63 in. open handle probe) within a given dietary treatment. Diets A, B, and C contained 127 ppm Zn, whereas, diets D, E, and F contained 121 ppm Zn.

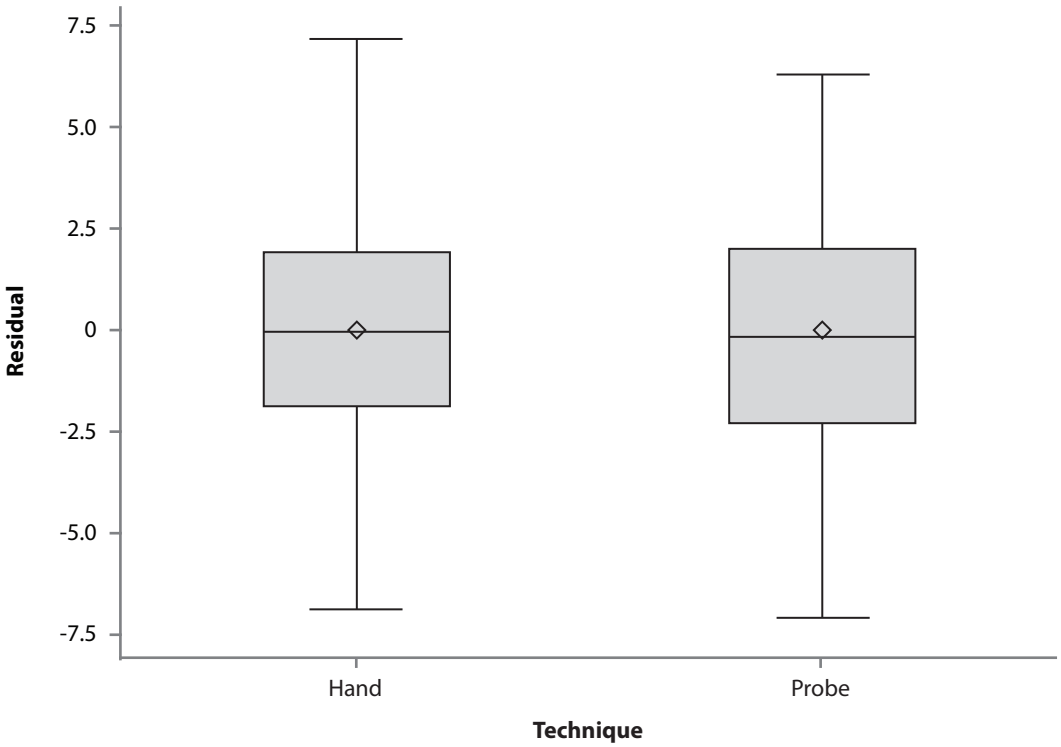

Figure 8. Statistical comparison examining the amount of variability attributed to samples obtained by hand and samples collected using a probe for the composite analysis of Zn.

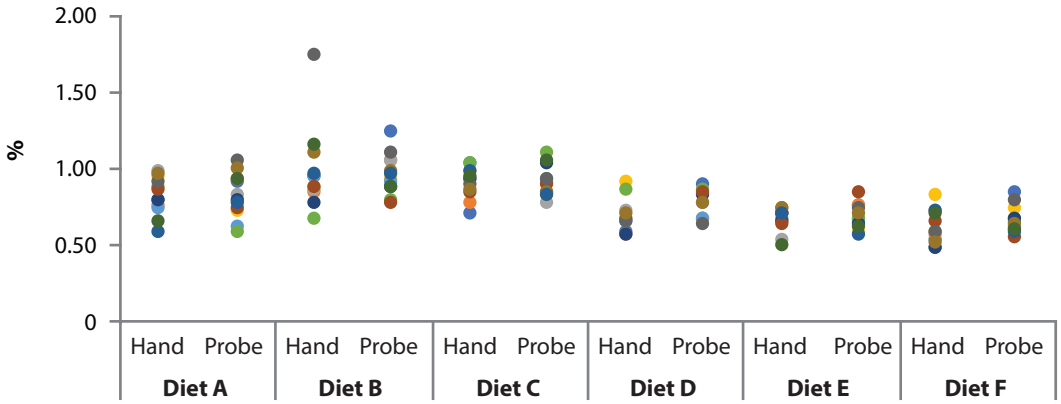

Figure 9. Distribution of analyzed mean Ca concentrations on an individual feeder basis. Each data point represents a single analysis of the 6 feeders in addition to its duplicate analysis for a total of 12 data observations for each sampling technique (hand: samples obtained by hand; probe: samples obtained using a 63 in. open handle probe) within a given dietary treatment. Diets A, B, and C contained 0.55% Ca; whereas, diets D, E, and F contained 0.50% Ca.

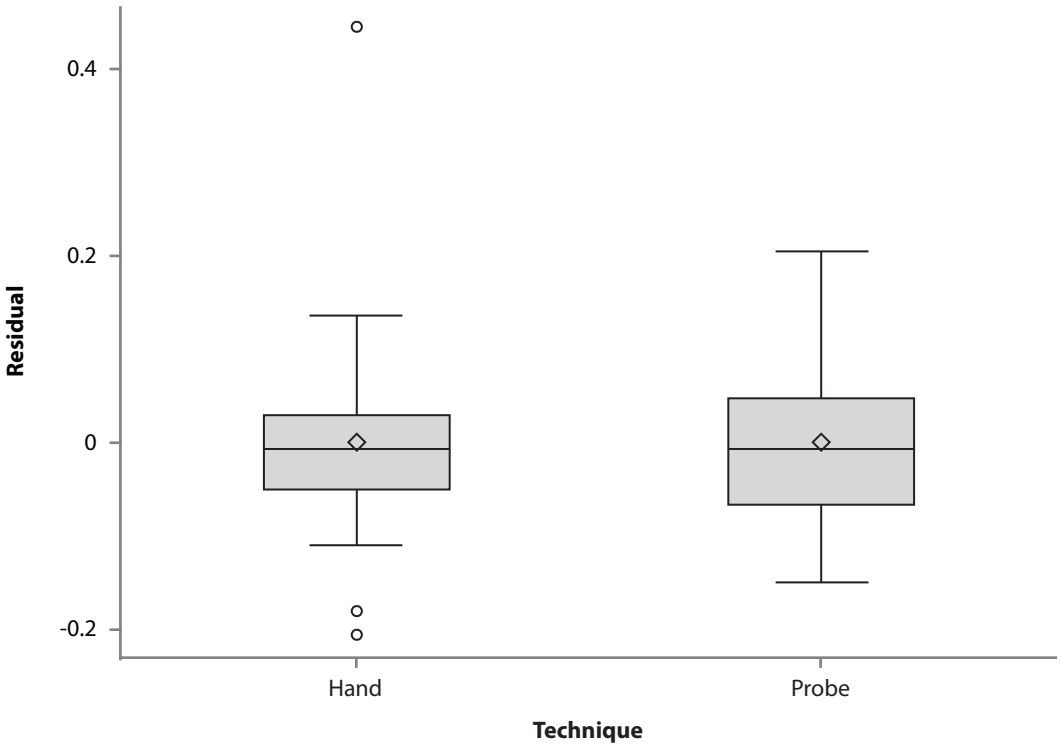

Figure 10. Statistical comparison examining the amount of variability attributed to samples obtained by hand and samples collected using a probe for Ca on an individual feeder basis.

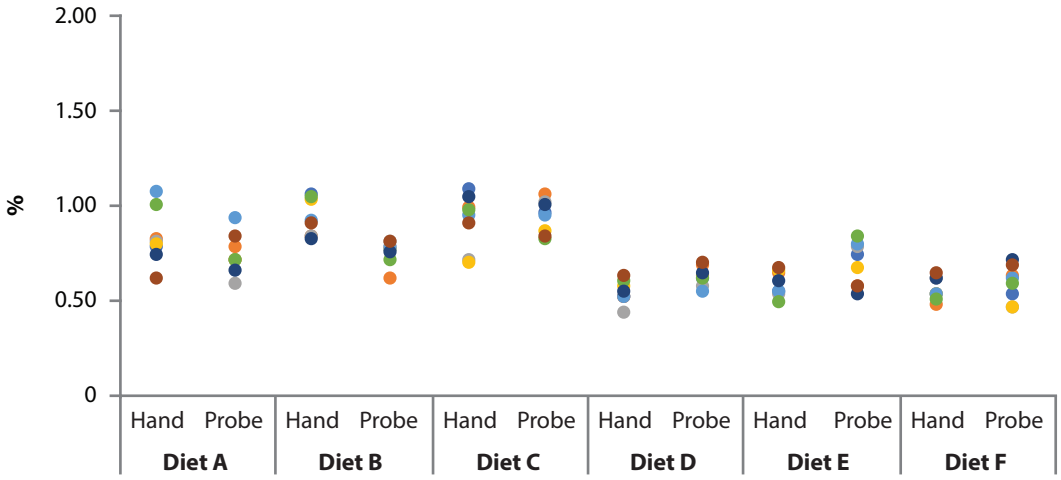

Figure 11. Distribution of analyzed mean Ca concentrations on a composite analysis basis in which subsamples from each of the 6 individual feeders were pooled within dietary treatment and sampling technique to form a single composite sample. The process was repeated until 4 individual composite samples were created for each diet and sampling technique. Each data point represents a single analysis on a composite sample in addition to its duplicate analysis for a total of 8 observations for each sampling technique (hand: samples obtained by hand; probe: samples obtained using a 63 in. open handle probe) within a given dietary treatment. Diets A, B, and C contained 0.55% Ca, whereas, diets D, E, and F contained 0.50% Ca.

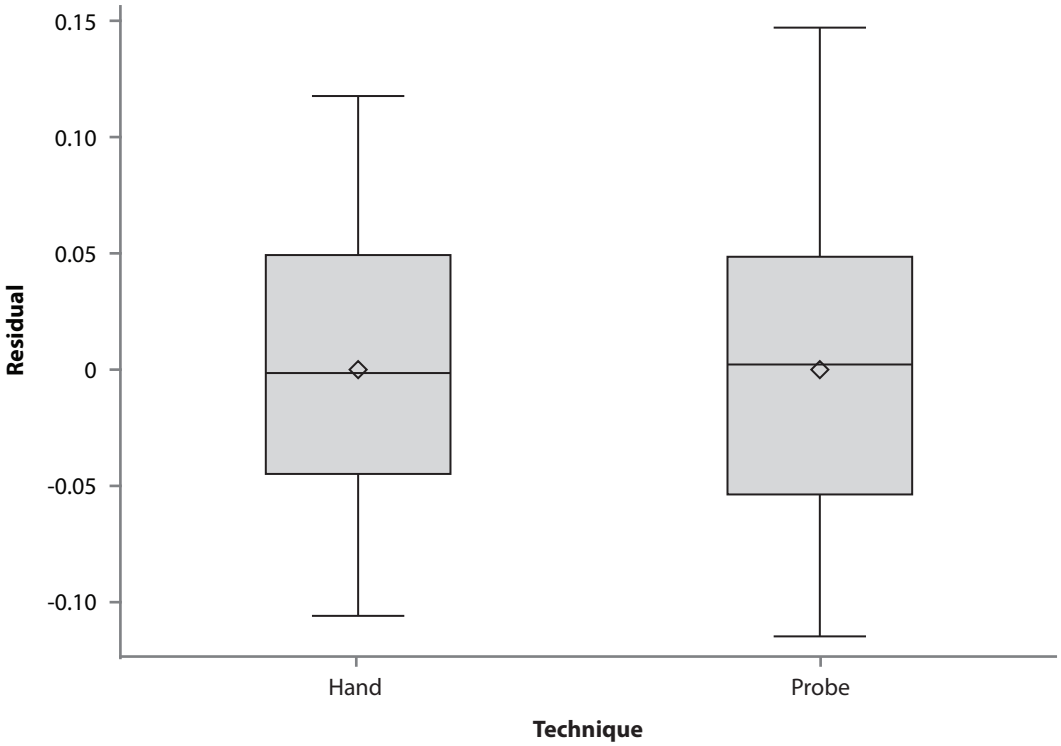

Figure 12. Statistical comparison examining the amount of variability attributed to samples obtained by hand and samples collected using a probe for the composite analysis of Ca.

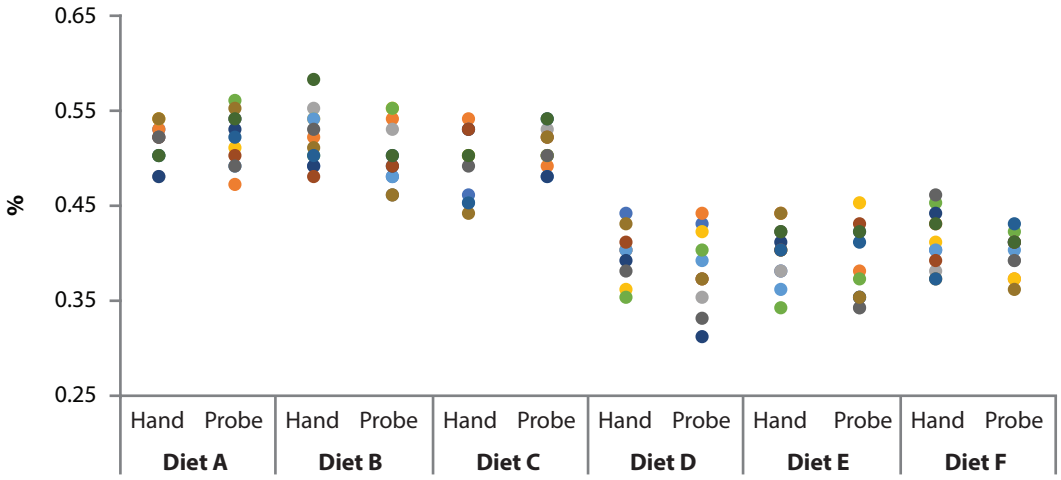

Figure 13. Distribution of analyzed mean P concentrations on an individual feeder basis. Each data point represents a single analysis of the 6 feeders in addition to its duplicate analysis for a total of 12 data observations for each sampling technique (hand: samples obtained by hand; probe: samples obtained using a 63 in. open handle probe) within a given dietary treatment. Diets A, B, C, D, E, and F contained 0.40% P.

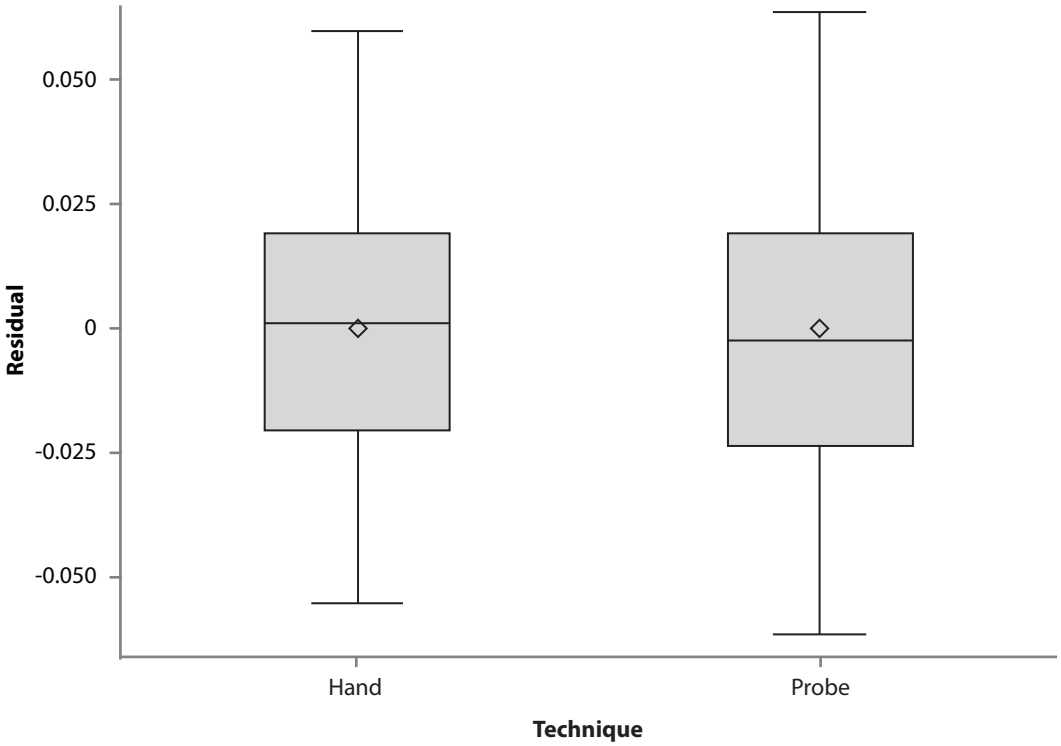

Figure 14. Statistical comparison examining the amount of variability attributed to samples obtained by hand and samples collected using a probe for P on an individual feeder basis.

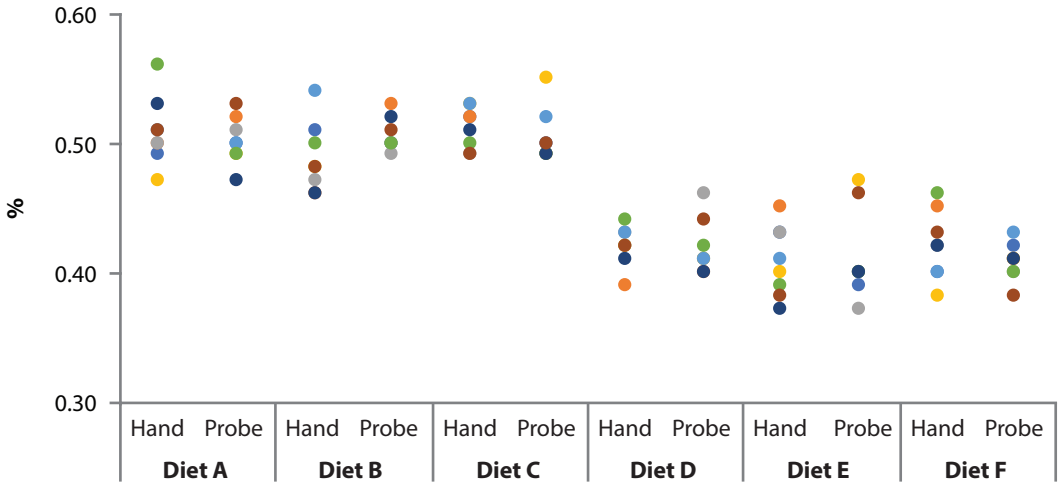

Figure 15. Distribution of analyzed mean P concentrations on a composite analysis basis in which subsamples from each of the 6 individual feeders were pooled within dietary treatment and sampling technique to form a single composite sample. The process was repeated until 4 individual composite samples were created for each diet and sampling technique. Each data point represents a single analysis on a composite sample in addition to its duplicate analysis for a total of 8 observations for each sampling technique (hand: samples obtained by hand; probe: samples obtained using a 63 in. open handle probe) within a given dietary treatment. Diets A, B, C, D, E, and F contained 0.40% P.

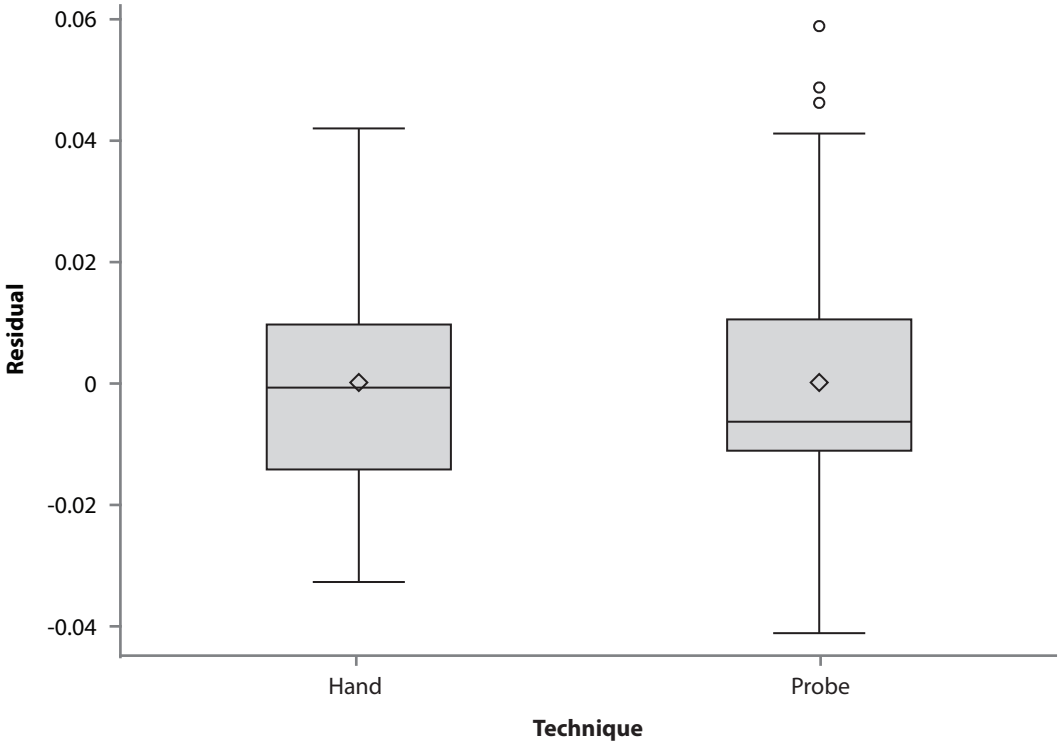

Figure 16. Statistical comparison examining the amount of variability attributed to samples obtained by hand and samples collected using a probe for the composite analysis of P.