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Assessing Error upon Sampling Bagged Feed

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ABSTRACT

The University of Kentucky Division of Regulatory Services explored the adequacy of the AOAC Official Method 965.16 for sampling bagged animal feed and assessed the acceptability of the sample error introduced through the sampling process. Eight feedstuffs were selected to represent a variety of feed forms, and their inherent differences in component size, with the objective of estimating the acceptability of the error introduced in the sampling process for each analyte. The findings suggest that the AOAC Official Method 965.16 is suitable for use in the collection of samples for regulatory purposes, particularly concerning mineral and drug components. The study's analysis and methodology provide valuable insights into the adequacy of the sampling method for regulatory purposes.

Keywords: agriculture, animal feed, analytical variability, measurement uncertainty, Association of American Feed Control Officials, AAFCO, sampling error, Horwitz, sampling

1. Introduction

The history of animal feed regulation is well documented in the Association of American Feed Control Officials (AAFCO) Official Publication (OP) (AAFCO, 2021). The birth of United States feed regulation began in 1895 with the first U.S. statute in Connecticut which charged the Connecticut Agricultural Experiment Station with enforcement of the feed law. In 1909, several additional states enacted analysis of food products and adulteration statutes, and other states were considering similar legislation. In 1909 the American Feed Manufacturers Association (AFMA) held a meeting with individuals representing feed manufacturers, retailers, and feed distributors in Washington DC. At the close of the meeting, control officials and industry representatives in attendance were asked to report "inspection and enforcement matters" (AAFCO, 2021) back to AFMA. Some of these feed control officials gathered for a follow up meeting to discuss feed regulation. This gathering of feed control officials marked the start of the Association of Feed Control Officials. This was later changed to Association of Feed Control Officials of the United States then a final name change (Association of American Feed Control Officials) to encompass other Western hemisphere nations. The association now also includes participation from Canada and Costa Rica (AAFCO, 2021).

A primary aspect of feed regulation involves assurance that products for sale are labeled in such a way that they are not misleading to consumers. This is evident in the core mission statement of AAFCO as follows: "A basic goal of AAFCO is to provide a mechanism for developing and implementing uniform and equitable laws, regulations, standards, definitions, and enforcement policies for regulating the manufacture, labeling, distribution and sale of animal feeds, resulting in safe, effective, and useful feeds. The association thereby promotes new ideas and innovative procedures and urges their adoption by member agencies, for uniformity" (AAFCO, 2021, 2009). This promotion of innovative procedures included the development of a uniform sampling technique. The laws and regulations regarding animal feed vary across the United States, as does enforcement, but the bagged feed sampling

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technique is uniform across AAFCO member states, taught at the AAFCO Basic Inspector Training Seminar, and published in the AAFCO Feed Inspectors Manual (AAFCO, 2017). The sampling method for bagged feed has followed a similar trajectory to the sampling of bagged fertilizers due to many of the foundational studies during the 1950s reporting on both sample types.

The 1929 Bibliography on Sampling of Fertilizers by R. N. Brackett recommended that individual cores would be taken from "not less than 10 per cent of the bags present" with additional qualifying information if the number of sampled bags was over 20 (Brackett, 1929). These individual cores were to be mixed and then reduced by quartering as documented in the sixth edition of the Official Methods of Analysis in 1945 (Midkiff, 1984). Later, Miles and Quackenbush recognized that the methods for sampling feed had been developed "in the absence of much basic information" (Miles & Quackenbush, 1950) and conducted studies to determine, through chemical analysis, the factors affecting the representativeness and precision in the sampling of fertilizer or feed using the Association of Official Analytical Chemists (AOAC) three-quarter inch trier (Midkiff, 1984). This trier was described at the time as a "standard single-tube trier which was pointed at one end, was slotted along one side, and had an inside diameter of three-quarters inch" (Miles & Quackenbush, 1950). The 6-state collaborative effort in 1950 (Miles & Quackenbush, 1950) to evaluate the sampling technique was followed by a 13-state collaboration in 1955 (Miles & Quackenbush, 1955), both conducted by Miles and Quackenbush. These multi-state collaborations included feed analytes protein, fat, fiber, and ash (Miles & Quackenbush, 1950), and culminated in the sampling technique currently used for sampling 50-pound bags of feed documented as the Association of Official Agricultural Chemists (AOAC) Official Method 965.16: the sampling of animal feed (AOAC, 2012c).

The feed sampling method originally instructed the inspector to "take cores from not less than 10% of bags but not more than 20 bags" and then thoroughly mix and mass reduce (Midkiff, 1984). The method was later modified to collect 10 probes (Spruill, 1965), composite in the field, and then conduct mass reduction. This mass reduction of the sample, either in the field or after receipt in the laboratory, can be achieved by hand-quartering, riffle-reducing, or some other mass reduction method. These reduction techniques have been previously evaluated in relationship to varying core numbers (Spruill, 1965). The riffle-reduced samples were determined to vary less from the true analysis determined from the composite sample when compared to the hand-quartering (Jeffers, 1940; Miles & Quackenbush, 1955) leading to the development of the current method. The previous studies involved multi-state sample collection and multiple laboratories for analysis, which potentially introduced additional variation. This variation could be reduced using a single state regulatory program with an in-house laboratory proficient in feed compliance testing and accredited by the International Organization for Standardization using 17025:2017 standards.

The early exploration of sampling error included analysis of protein, fat, fiber, and ash which developed AOAC Official Method 965.16 for sampling. The University of Kentucky Division of Regulatory Services, which administers Kentucky feed law and regulations (KRS 250.491), examined whether AOAC Official Method 965.16 was adequate for sampling animal feed to test for other analytes such as minerals and drugs, for compliance. We hypothesize that feed samples collected by AOAC method 965.16 for minerals and drugs will result in acceptable levels of variation, and the sampling method should be suitable in the collection of samples for regulatory purposes that include the analysis of mineral and drug components.

2. Materials and Methods

Eight feedstuffs, commonly collected using the AOAC Official Method 965.16 for sampling, were selected to represent a variety of the feed forms available today and their inherent differences in component size (Table 1 in Appendix). The selection included a standard beef mineral (Feed 1) to represent a loose or granular feed with higher mineral concentrations. The layer complete feed (Feed 2) was chosen to exemplify a meal form feed. The beef commodity mix (Feed 3) containing molasses and exhibiting significant particle size differences represents a typical range of minerals seen in the feed regulatory setting. The pelleted soybean meal (Feed 4), a single-ingredient feed is representative of common commodities used in the manufacture of animal feeds. The sheep complete medicated feed (Feed 5), a pelleted feed, was included to evaluate a drug analyte. The dried distiller's grains (Feed 6), another single ingredient feed, represents common commodities used in the manufacture of animal feeds. The gamebird feed (Feed 7), a textured feed without molasses, with varying particle sizes and typical mineral concentrations, was included as it is frequently seen in Kentucky. Lastly, an extruded dog food sample (Feed 8) with a suitable kibble size, was included as a feed form sampled using the AOAC ¾" trier as per the AOAC Official Method 965.16 for sampling. The objective of selecting these feed types was to provide a value for the error introduced by the sampling process for each analyte, enabling regulatory bodies to determine the acceptability of this error within their regulatory frameworks. Feed types 4 and 6 (soybean meal and dried distiller's grains) were excluded from mineral analysis due to their inherently low values falling below the method's lower limit of detection.

To determine if components of an animal feed are accurately reported through sampling, testing, and reporting, there are unavoidable errors surrounding the process that must be taken into consideration. This error is expected and accounted for by state regulatory bodies to determine compliance through the use of investigational allowances or analytical variations. If an analyte result is not within a positive or negative investigational allowance (as determined by each individual regulatory program) from the guarantee, then the analyte may be considered violative for reasons beyond the unavoidable error calculated for that analyte. Analytical variations (AVs) published by AAFCO have been used by regulatory bodies directly or indirectly to assist in the determination of regulatory compliance. However, the data and statistical theories behind the development of the AAFCO values have changed over time and the values are not to be used directly as investigational allowances. The purpose of the AAFCO AVs are to provide between-lab variance among laboratories when analyzing the same material for the same analytes, at the same concentration, using the same method on a ground sample (AAFCO, 2021). Although no specific details exist on the statistical methods used to develop the AVs, it is believed to be derived from interlaboratory variability from ground and well-homogenized proficiency testing samples.

To calculate the combination of unavoidable errors, one must first identify sources of error. There are three primary sources of error that include sampling error, sample preparation error, and laboratory error. Table 2 in Appendix shows an overview of the errors associated with sampling and testing feed. First, the error associated with the process of obtaining the sample is identified as sampling error or sampling relative standard deviation (RSD). Second, sample reduction variation, or the error encountered during mass reduction for preparing an analytical sample for laboratory analysis, is identified as sample preparation error (Prep RSD). Third, laboratory error (Lab RSD) encompasses error in selecting the test portion from a ground analytical sample and error in the chemical analysis. These errors can vary greatly based on the methods used, concentration of the analyte, and sample type. The Global Estimation Error (Global RSD) is the square root of the sum of the individual error RSD values squared.

The sampling RSD includes material heterogeneity error, and error surrounding the sampling process itself through the collection of probed cores and subsequent compositing. Material heterogeneity was controlled to the extent that it could be by the feed manufacturer. Manufacturing facilities were selected that followed good manufacturing practices as established by FDA in 21 CFR Part 507 to ensure correct handling and processing of animal feed so material heterogeneity was typical of good manufacturing firms. In addition, firms in Kentucky were selected based on the capability to produce the targeted feedstuffs for the study and had a mixer capacity minimum of 3 tons. To minimize the potential for additional unintended error, all samples were collected by a University of Kentucky inspector trained and audited to sample per the AAFCO method for bagged feeds. This method involves the diagonal insertion of the ¾" single tube trier into the bag with the slot facing down. Once the trier has been inserted the inspector turns the trier face upwards. The filled trier is then withdrawn, and the sample core deposited into a pre-labeled sample bag. The AAFCO method for sampling states that at least 10 sample cores be taken from 10 50-pound bags in the same lot and composited. For this study, each sample core was placed in its own sample bag for analysis. Feed lots with at least 40 50-pound bags were targeted so 40 individual sample cores from separate bags were taken for laboratory analysis for each feed type. The Prep RSD due to sample reduction error was nonexistent since the sample cores were not composited and no mass reduction occurred to create an analytical sample. Each individual core sample was approximately 200 g and was ground using a Retsch ZM1000 Grinder with a 0.75 mm screen. The ground analytical samples were placed in pre-labeled sample jars for further analytical testing.

All core samples were analyzed individually using approved AOAC Methods of Analysis (MOA) where applicable. These methods shown in Table 3 include AOAC MOA 990.03 for crude protein in animal feed by combustion (AOAC International, 2012b), AOAC MOA 2017.02 simultaneous determination of mineral content by microwave acid digestion and inductively coupled plasma-optical emission spectrometry detection (Webb, 2018), and AOAC MOA 2008.01 Lasalocid sodium in animal feeds and premixes (AOAC International, 2012a). During AOAC MOA 2017.02 microwave acid digestion, strategic organization of the digestion tubes within the microwave oven was utilized to minimize and distribute any microwave set variability across sample types.

Quantifying errors due to material heterogeneity and sampling process as components of sample error (AAFCO, 2015, 2018; Wagner & Esbensen, 2015) was beyond the scope of this study. The goal of this study was not to identify various components of sampling error in the various feed types. Rather, the goal was to estimate and evaluate acceptability of the sample error involving the sampling process as it is currently conducted across a wide range of feed materials for minerals and drug components when compositing 10 cores as per AOAC method 965.16. In this study, test results from 10 individual cores were averaged to represent a sample result as if collected and composited according to AOAC method 965.16. From 40 individual core results, four average results (from the four groups of 10 cores each) were analyzed to determine the global RSD.

With 40 individual core results, there are 847,660,528 possible combinations of 10 probes from 40 bags as determined by 40! / 10! x (40 - 10)!. Therefore, if an inspector randomly chose 10 of 40 bags to probe there would be more than 847 million possible samples. Two possibilities of four samples were considered for determining average and standard deviation in Figures 1a through 1k. One possibility was a worst-case scenario where individual core test results were sorted from low to high concentrations and four groups of 10 results were selected from the sequential order from low to high concentrations. The probability of these four samples being obtained from sampling and homogenizing 10 cores for each sample is extremely rare. Probing 10 bags out of the 40 with the lowest (or highest) analyte values to create a composite sample was deemed the worst-case scenario in sampling variability when obtaining a sample from 40 bags. Another possibility considered was a randomization of the 40 probes to obtain 4 random composites of 10 cores each that would more closely represent 4 samples collected by the sampling method. A single random assortment of the 40-probe data into four groups is only representative of that particular selection of four groups. Many more possible sets of four random groups could be selected. With this in mind, five sets of four random groups (containing 10 probes each) were obtained from the 40-probe data. An analysis was then made on global RSD from the 40 individual core data and the five random data sets with global RSD plotted versus analyte concentration in Figure 2 in the Appendix. The graphs also contain the AAFCO AV RSD and Horwitz RSD for reference and comparison. The AAFCO AVs, previously identified as Permitted Analytical Variations (PAVs), were derived from interlaboratory variation of proficiency test samples being 2 times the interlaboratory RSD (AAFCO, 1985). The AV RSD was then determined as:

$$\text{AV RSD} = \text{AV\%} / 2$$

The Horowitz RSD is an expected estimate of variation across laboratories analyzing ground and homogenized material. The Horwitz RSD (Rivera et al., 2011) was determined as follows:

$$\text{For } C <= 0.138\text{: Horwitz RSD} = (0.02 \times C \wedge 0.8495) / C \times 100$$

$$\text{For } C > 0.138\text{: Horwitz RSD} = (0.01 \times C \wedge 0.5) / C \times 100$$

where C is a dimensionless concentration. For analytes with concentration in %, C = analyte concentration / 100. For analytes with concentration in ppm, C = analyte concentration / 1,000,000.

The RSD of results from various combinations of 4 composite samples of the individual cores represents the global estimation error which includes sampling error, prep error and laboratory error (Table 2 in Appendix). The prep error is nonexistent since no mass reduction occurred for each core sample taken. Laboratory error was assumed to be smaller than sampling error, so global RSD was assumed to be a good approximation of sampling RSD. Even if laboratory RSD was as much as half of sampling RSD, global RSD would only be 12% greater than compared to a situation with the same sampling RSD and laboratory RSD being 0%. The modest increase in global RSD is due to global RSD being the square root of the sum of squares of individual component RSDs. Another way to view the global RSD values determined from the study is they represent the maximum expected sampling RSD. Sampling RSD could not be greater than global RSD.

3. Results and Discussion

By designing the study to remove or minimize known sources of error, focus was placed on error introduced through sampling the feed product to determine if the AOAC bagged feed sampling method is appropriate for use in a regulatory capacity for mineral analytes and drugs.

In the scatterplots showing the feed type analyte composites compared with the AAFCO AV (Figures 1a through 1k in Appendix) the average from the 40 individual cores is assumed to be the average for the composite possibilities with 1 and 2 standard deviations from the average plotted. The 40 individual probe values are displayed by blue points. The orange points show the worst-case scenario of four composite samples where each value is an average of a ten-probe composite with the individual prove values sorted low to high concentrations prior to compositing. The four gray points are each an average of 10 probe values where four sets of 10 probe values were selected from a randomization of the 40 probe values. Most, if not all, regulatory bodies use the AAFCO AV table in some way, either in their calculations or to directly determine regulatory limits. With this in mind, we then overlaid the data with the current AAFCO AVs for the analytes that have a defined AV: crude protein, calcium, phosphorous, magnesium, sodium, potassium, copper, iron, manganese, zinc, selenium, and lasalocid. The AAFCO AVs are intended to be applied to the analyte guarantee as the 95% confidence interval for a single analyte analysis result, calculated from the AAFCO check sample program. For the AAFCO AV calculations we used the 40-probe analyte mean, as the value to be most true, or the closest value known to the actual value.

The variability of the four composited samples sorted low to high is similar to the variability of the 40 individual cores; however, a significant reduction in variability occurs with the random composite of 10 probes, as would most likely occur following the AAFCO feed sampling method for bagged feeds. The variability of the 40-core data appears to be normally distributed for most analytes. Exceptions to normal distribution include manganese and zinc for the beef commodity mix (Feed 3), (Figures 1i and 1j in Appendix). Manganese is not normally distributed in the 40-probe data and shows greater variability in the composite data. Exceptions to normality and increases in variability were expected to be seen in the less heterogeneous feed types like the beef commodity mix and the game bird feed. The game bird feed also showed exceptions to normality in manganese and zinc (Figures 1i and 1j in Appendix), but random composite samples decreased the variability in these analytes. Herrman et al. (2022) noted many of the AAFCO mineral AV's were shown to be "applicable for their intended purpose" with the exception of cobalt and selenium (Herrman et al., 2022). Our data on selenium is limited due to only the beef mineral (Feed 1) having levels above the laboratory level of quantification (LOQ). The selenium 40 probe data, and the data sorted low to high falls above and below the AAFCO AV, but the variability again decreases with the random composite and is well within the AV (Figure 1k). The ±2 standard deviations were greater than or equal to the AAFCO AVs in 29 of the 63 cases of analyte and feed type in the 40-probe data (Figures 1a through 1k in Appendix). However, only protein in Feed 3 out of the 63 cases of the randomized composites had ±2 standard deviations greater than or equal to the AAFCO AVs.

Table 1 in Appendix shows the values from 4 random composites including mean, standard deviation, analyte's AAFCO AV, calculated mean ± one and two times the standard deviation (lower and upper values shown), and the calculated lower and upper values for the AV limits of the mean.

The relationship between analyte concentration and the relative standard deviation for each analyte is plotted along with the AAFCO AV (black line) and the calculated Horwitz RSD (yellow line) in Figure 2 in Appendix. The square data points with the feed type number represent the RSD result from the 40 individual cores. The vertical lines directly below the numbered squares correspond to RSDs from 5 sets of 4 randomized composites showing the low, average, and high values. Trends are similar to data in Figure 1. Both the 40 individual core data and the composite data show variability; however, the composite data contains significantly less variability than the individual cores. The feed types with expected high variability due to noticeable particle size differences, the beef commodity mix (Feed 3) and the gamebird feed (Feed 7), both show high variability for protein in their 40 individual core data RSD. The soybean meal revealed an unexpectedly higher RSD in the 40 individual core data, but as with the other feed types, was consistently less variable in the composite data.

The global RSD values for the four randomized composites was an estimate of sampling error using the AOAC method of taking 10 cores and compositing to obtain a sample. In all cases, the four randomized composites resulted in significant reductions in global RSD compared to the RSD of the 40 individual probes. Although there were some exceptions, the RSD values were significantly less than the AAFCO AV RSD and Horwitz RSDs. The RSD values were also far less than the 35% error mentioned as an upper limit for making an inference on material sampled (AAFCO, 2015). The low RSDs indicate the AAFCO method of sampling taking 10 cores and compositing was adequate for the analytes tested.

4. Conclusion

The goal of this study was to assess the suitability of the AOAC official method 965.16 sampling of animal feed as an acceptable method for sample collection when the scope includes minerals and animal drugs. We found that feed samples collected by this method to contain acceptable levels of variation for mineral and drug components and the error surrounding these analytes is tolerable. We believe that the AOAC 965.16 official sampling method should be extended and regarded as suitable for use in the collection of samples for regulatory purposes. The global RSD values were significantly less than AAFCO AV RSDs and Horwitz RSDs indicating the suitability of compositing 10 cores as a representative sample. Additionally, the RSD from 4 random composite values can be taken as an estimate of sampling error to be included in a laboratory's calculation of measurement uncertainty if adjustments have not already been made in the regulatory decision-making process to account for sampling error.

As eloquently stated in the Theory of Sampling Applied to Food Safety and Environmental Protection, "If the test results from the laboratory are not representative of the average concentration, they are useless for estimating risk" (Ramsey et al., 2019). Based on the data presented here we conclude that a representative sample can be collected from 50-pound bags using the current AAFCO sampling method for the analysis of minerals and drug components.

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Appendix – Table and Figures

Table 1: Eight feed types studied with the feed form description, analytes analyzed, and image of each feed.

		1 Beef mineral	2 Layer complete	3 Beef commodity mix	4 Soybean meal	5 Sheep complete medicated	6 Dried distillers grains	7 Gamebird feed	8 Dog food
Feed Form	Loose or granular	M							
	Textured with molasses			CP, M					
	Pelleted, Single Ingredient				CP				
	Pelleted, Medicated Feed					CP, M, Med.			
	Meal		CP, M						
	Meal, Single Ingredient						CP		
	Textured without molasses							CP, M	
	Extruded								CP, M
	Image of feed								

Analyte(s) run: Crude Protein (CP), Minerals (M), Drug - Lasalocid (Med.)

Table 2: Summary of errors in sampling feed.

Global Estimation Error (Global RSD) $(\text{Global RSD})^2 = (\text{Sampling RSD})^2 + (\text{Prep RSD})^2 + (\text{Lab RSD})^2$					
Sampling Error (Sampling RSD)			Sample Prep Error (Prep RSD)	Laboratory Error (Lab RSD)	
Feed (Material Heterogeneity)	Probed Cores	Compositing	Reduction of Composite Sample in Lab	Obtaining a test portion	Chemical Analysis

Table 3: AOAC methods used (AOAC International, 2012a, 2012b; Webb, 2018). Analytes are shown with their unit of measure, AOAC reference, and the limit of quantification (LOQ)

Analyte	AOAC Reference	LOQ
Crude Protein (%)	990.03	0.16
Ca (%)	2017.02	0.0022
Co (ppm)	2017.02	0.7
Cu (ppm)	2017.02	1.65
Fe (ppm)	2017.02	10
Mg (%)	2017.02	0.0010
Mn (ppm)	2017.02	0.72
P (%)	2017.02	0.00049
K (%)	2017.02	0.00050
Na (%)	2017.02	0.00067
Selenium (ppm)	2017.02	0.96
Zn (ppm)	2017.02	5.09
Lasalocid (ppb)	2008.01	1.09

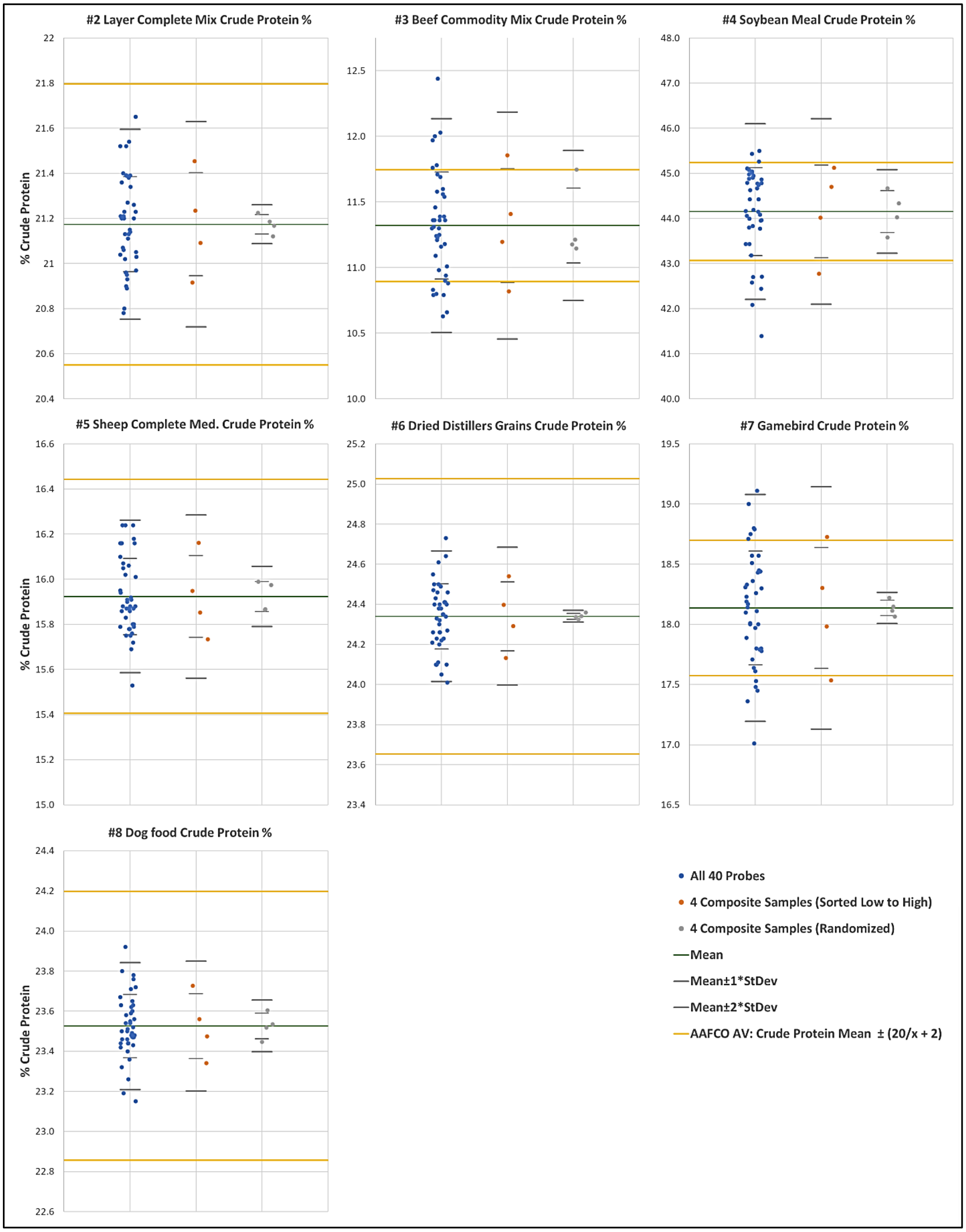

Figure 1a: Crude protein results from 40 core samples of seven feed types. Data shown for all individual cores, worst-case scenario of four composite samples of 10 cores per sample determined from 40 cores ranked from low to high concentration, and a randomized selection of four composite samples of 10 cores per sample from the 40 cores. Mean from the 40 individual core results and AAFCO AV of that mean are also shown for comparison.

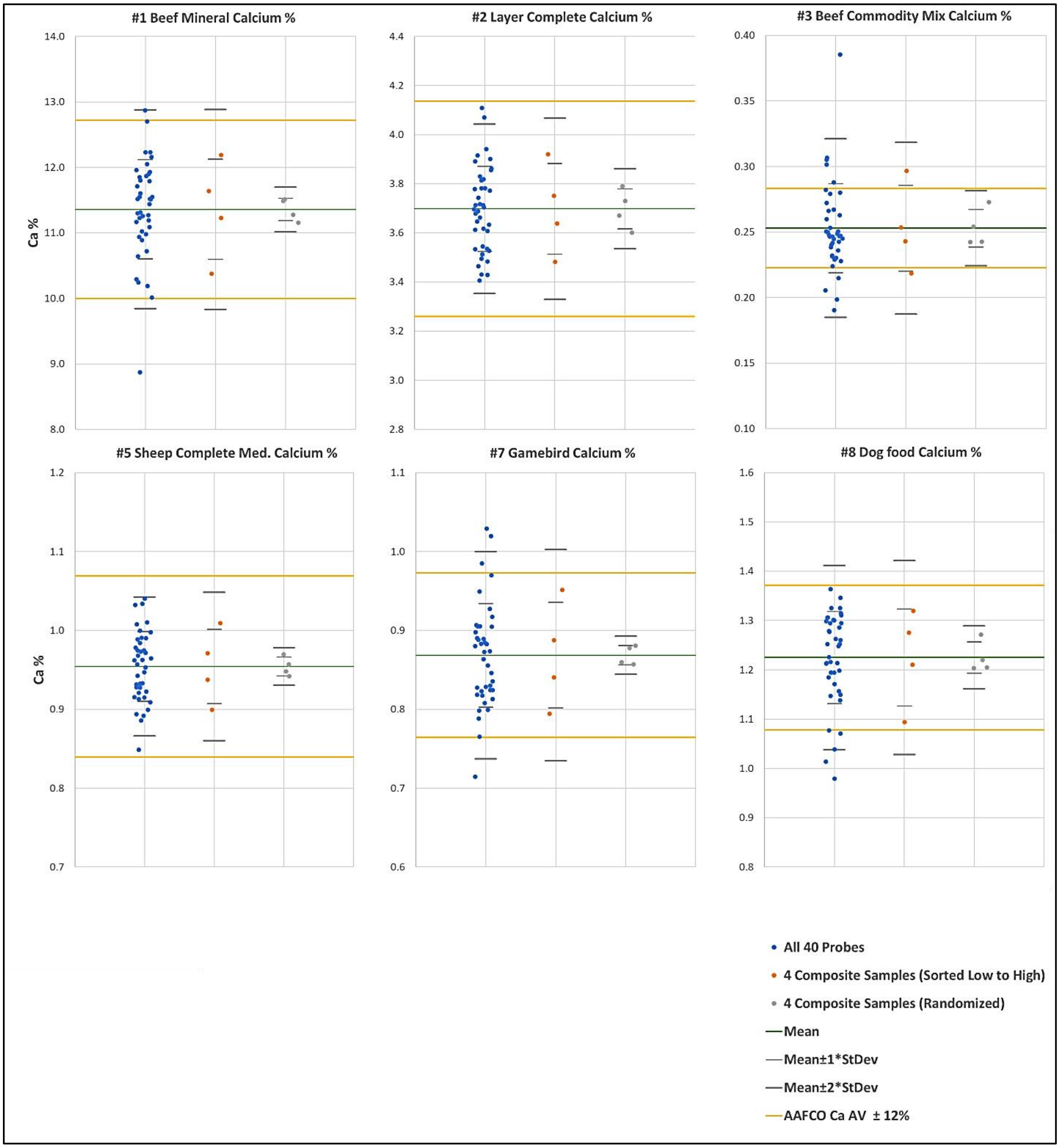

Figure 1b. Calcium results from 40 core samples of six feed types. Data shown for all individual cores, worst-case scenario of 4 composite samples of 10 cores per sample determined from 40 cores ranked from low to high concentration, and a randomized selection of 4 composite samples of 10 cores per sample from the 40 cores. Mean from the 40 individual core results and AAFCO AV of that mean are also shown for comparison.

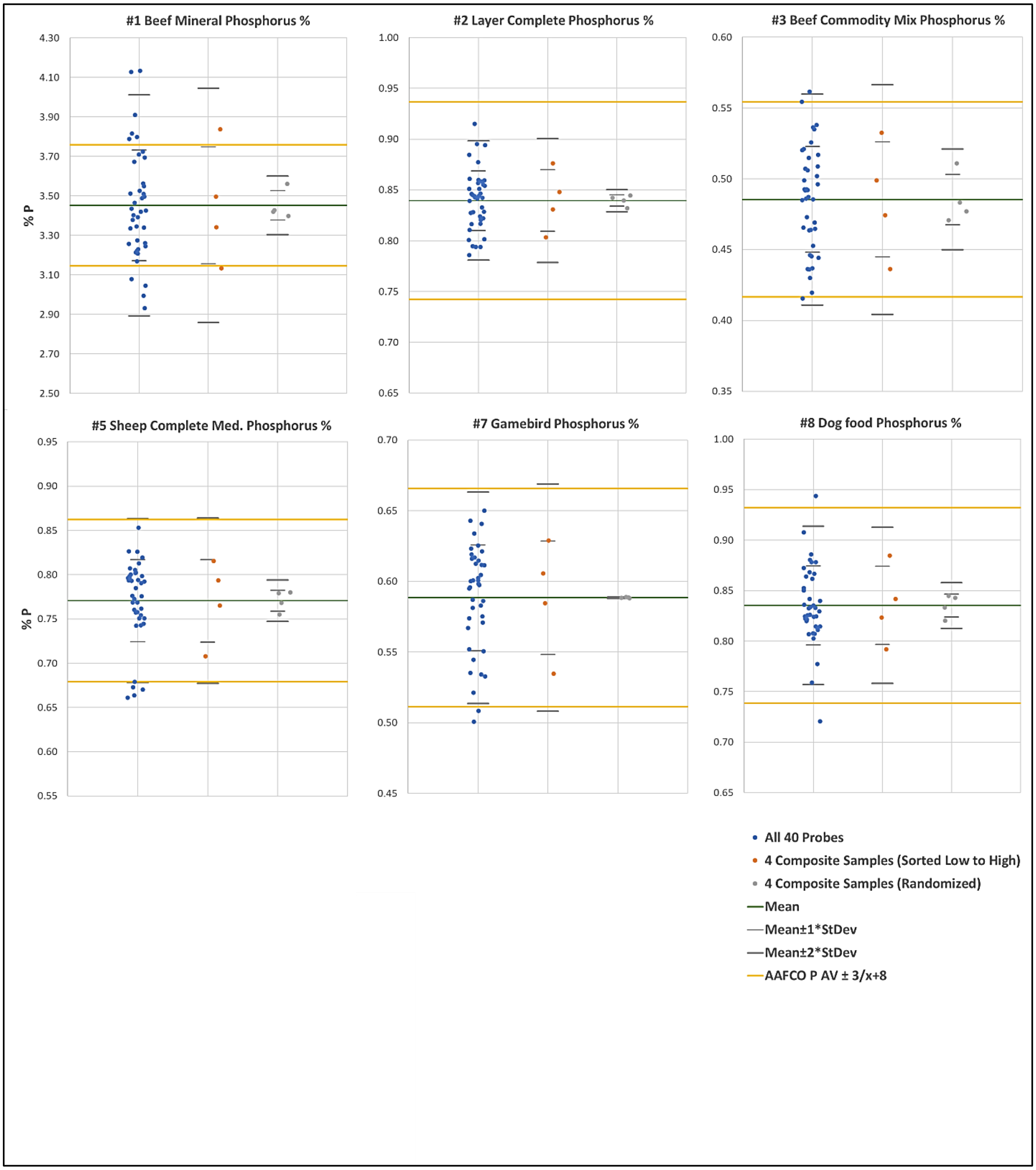

Figure 1c. Phosphorus results from 40 core samples of six feed types. Data shown for all individual cores, worst-case scenario of 4 composite samples of 10 cores per sample determined from 40 cores ranked from low to high concentration, and a randomized selection of 4 composite samples of 10 cores per sample from the 40 cores. Mean from the 40 individual core results and AAFCO AV of that mean are also shown for comparison.

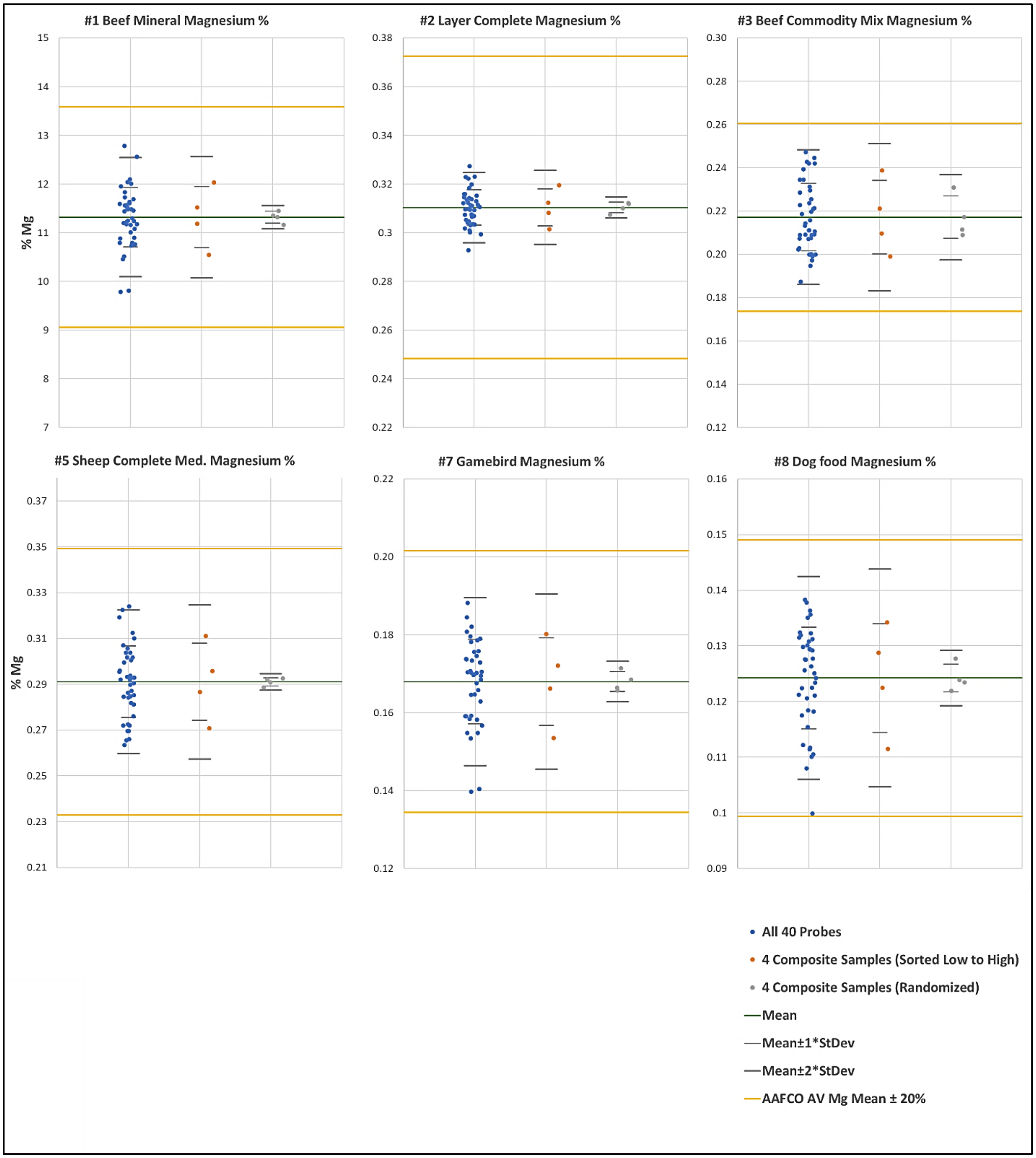

Figure 1d. Magnesium results from 40 core samples of six feed types. Data shown for all individual cores, worst-case scenario of 4 composite samples of 10 cores per sample determined from 40 cores ranked from low to high concentration, and a randomized selection of 4 composite samples of 10 cores per sample from the 40 cores. Mean from the 40 individual core results and AAFCO AV of that mean are also shown for comparison.

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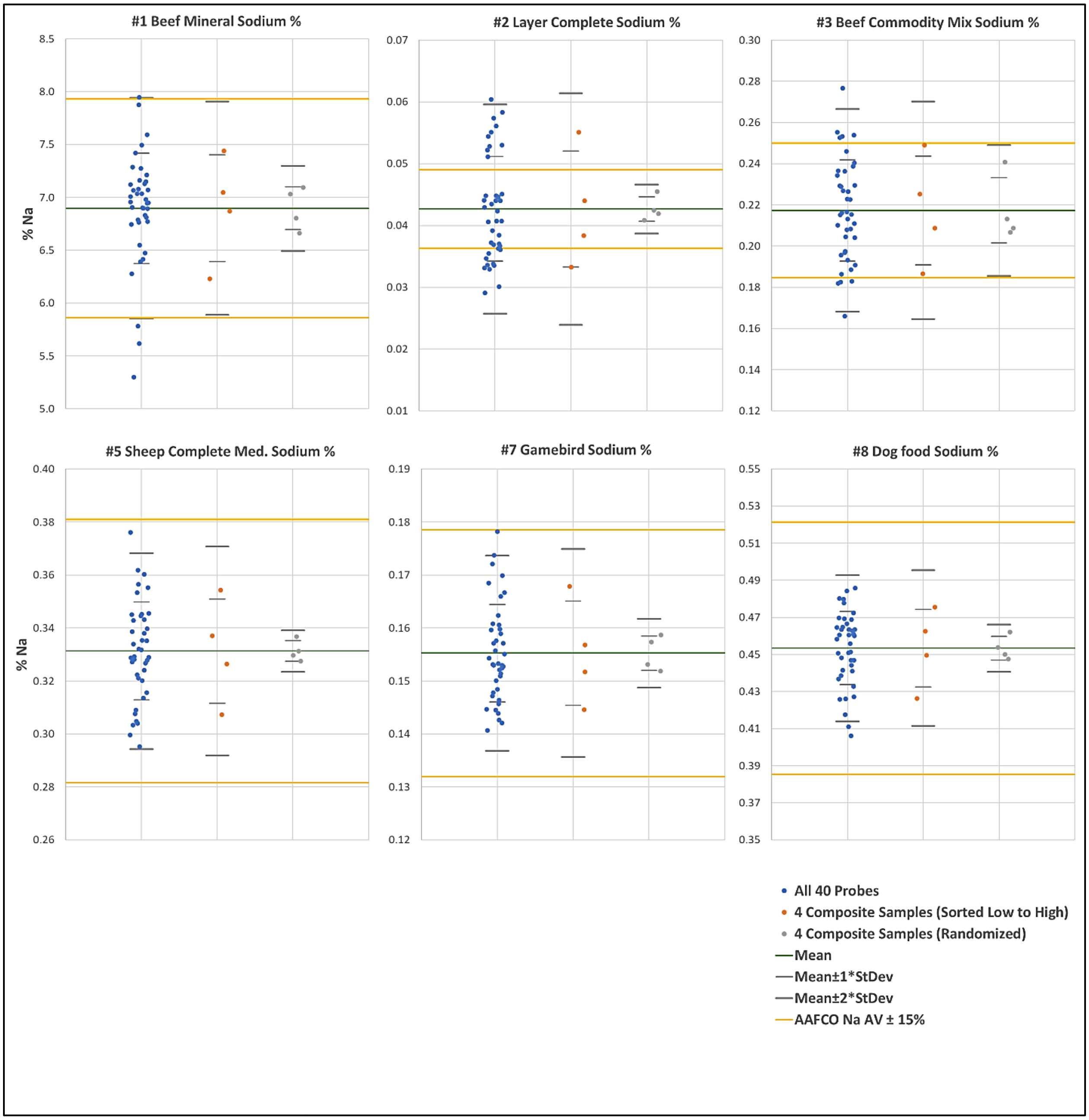

Figure 1e. Sodium results from 40 core samples of six feed types. Data shown for all individual cores, worst-case scenario of 4 composite samples of 10 cores per sample determined from 40 cores ranked from low to high concentration, and a randomized selection of 4 composite samples of 10 cores per sample from the 40 cores. Mean from the 40 individual core results and AAFCO AV of that mean are also shown for comparison

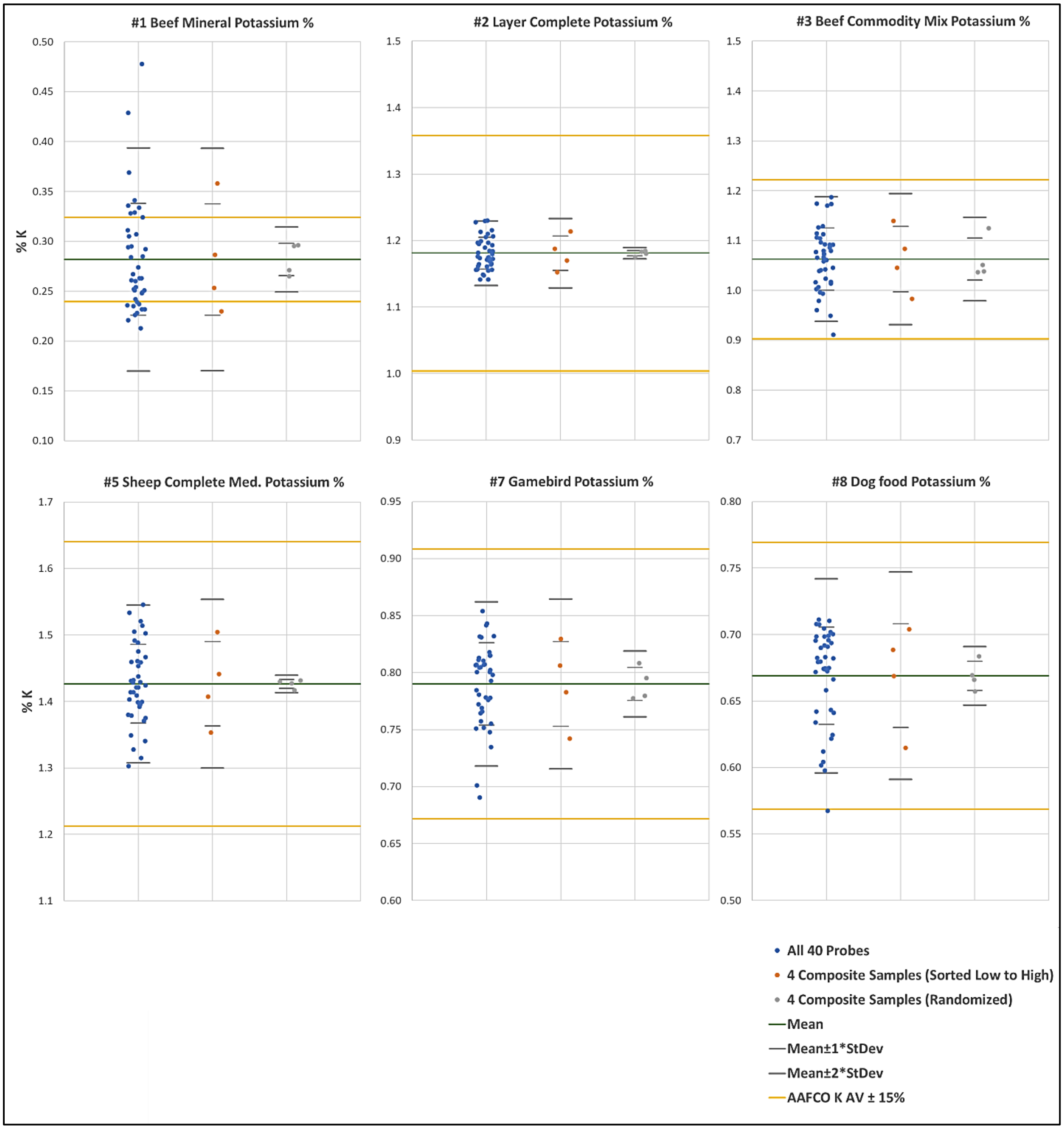

Figure 1f. Potassium results from 40 core samples of six feed types. Data shown for all individual cores, worst-case scenario of 4 composite samples of 10 cores per sample determined from 40 cores ranked from low to high concentration, and a randomized selection of 4 composite samples of 10 cores per sample from the 40 cores. Mean from the 40 individual core results and AAFCO AV of that mean are also shown for comparison.

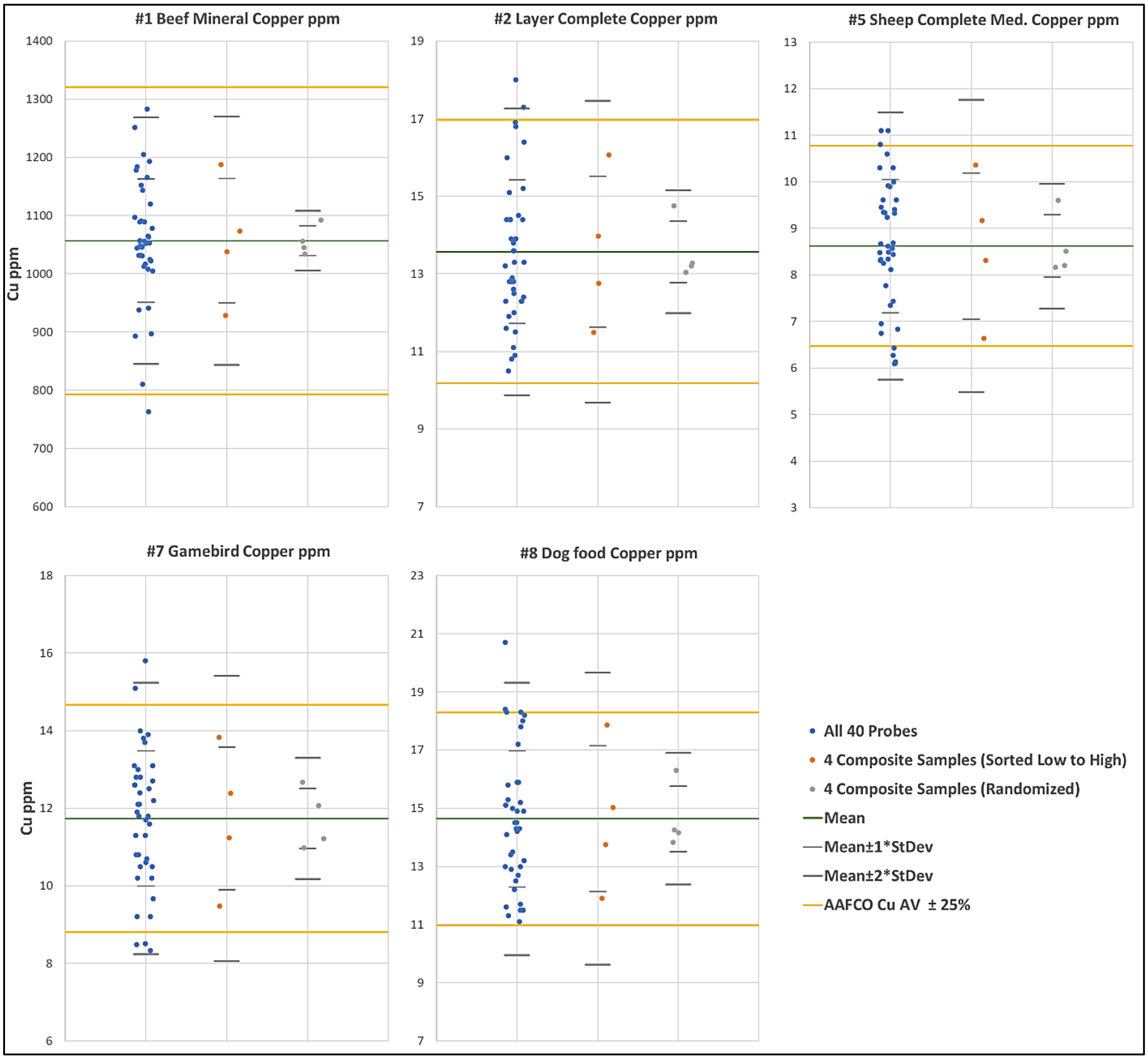

Figure 1g. Copper results from 40 core samples of five feed types. Data shown for all individual cores, worst-case scenario of 4 composite samples of 10 cores per sample determined from 40 cores ranked from low to high concentration, and a randomized selection of 4 composite samples of 10 cores per sample from the 40 cores. Mean from the 40 individual core results and AAFCO AV of that mean are also shown for comparison.

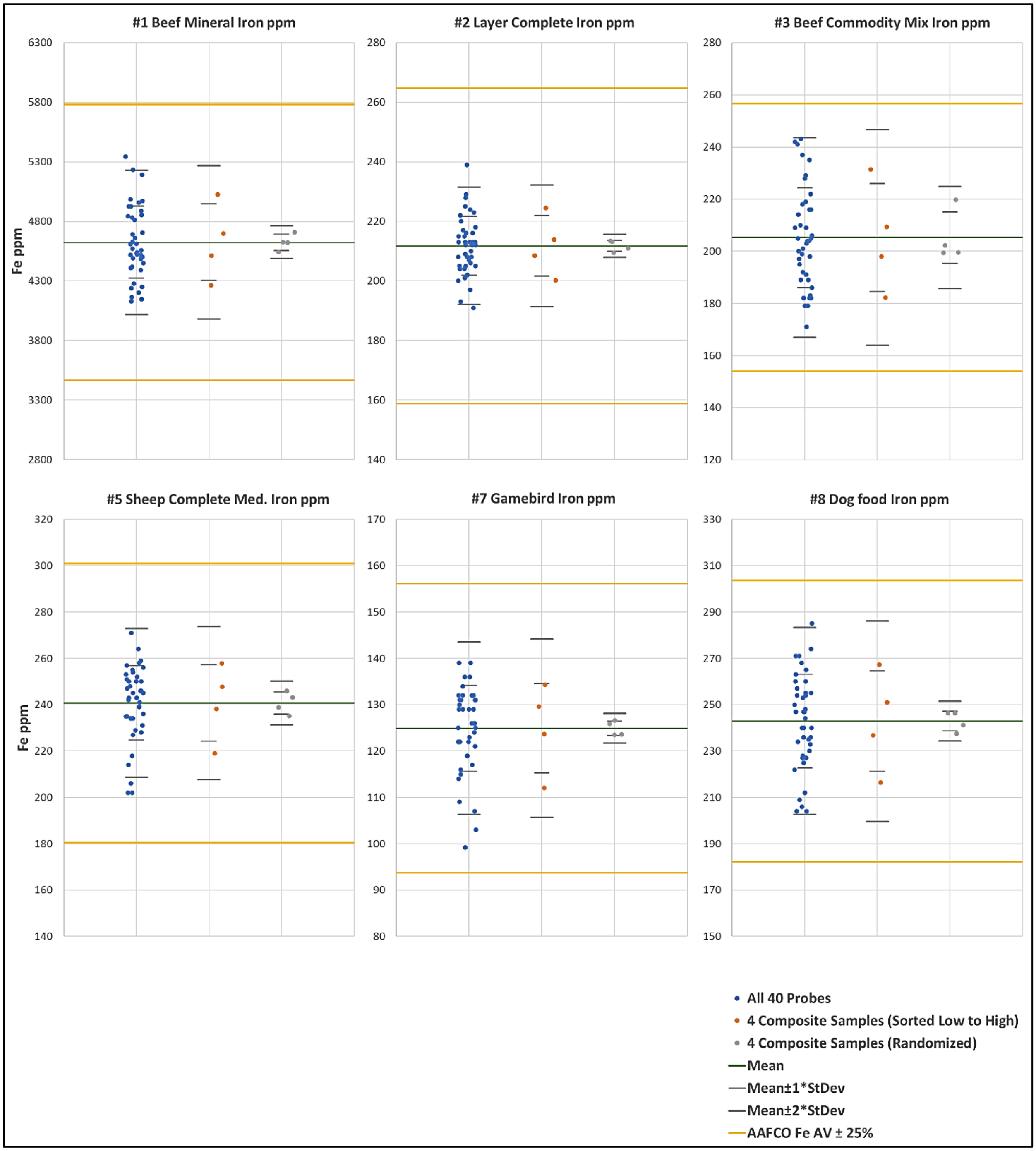

Figure 1h. Iron results from 40 core samples of six feed types. Data shown for all individual cores, worst-case scenario of 4 composite samples of 10 cores per sample determined from 40 cores ranked from low to high concentration, and a randomized selection of 4 composite samples of 10 cores per sample from the 40 cores. Mean from the 40 individual core results and AAFCO AV of that mean are also shown for comparison.

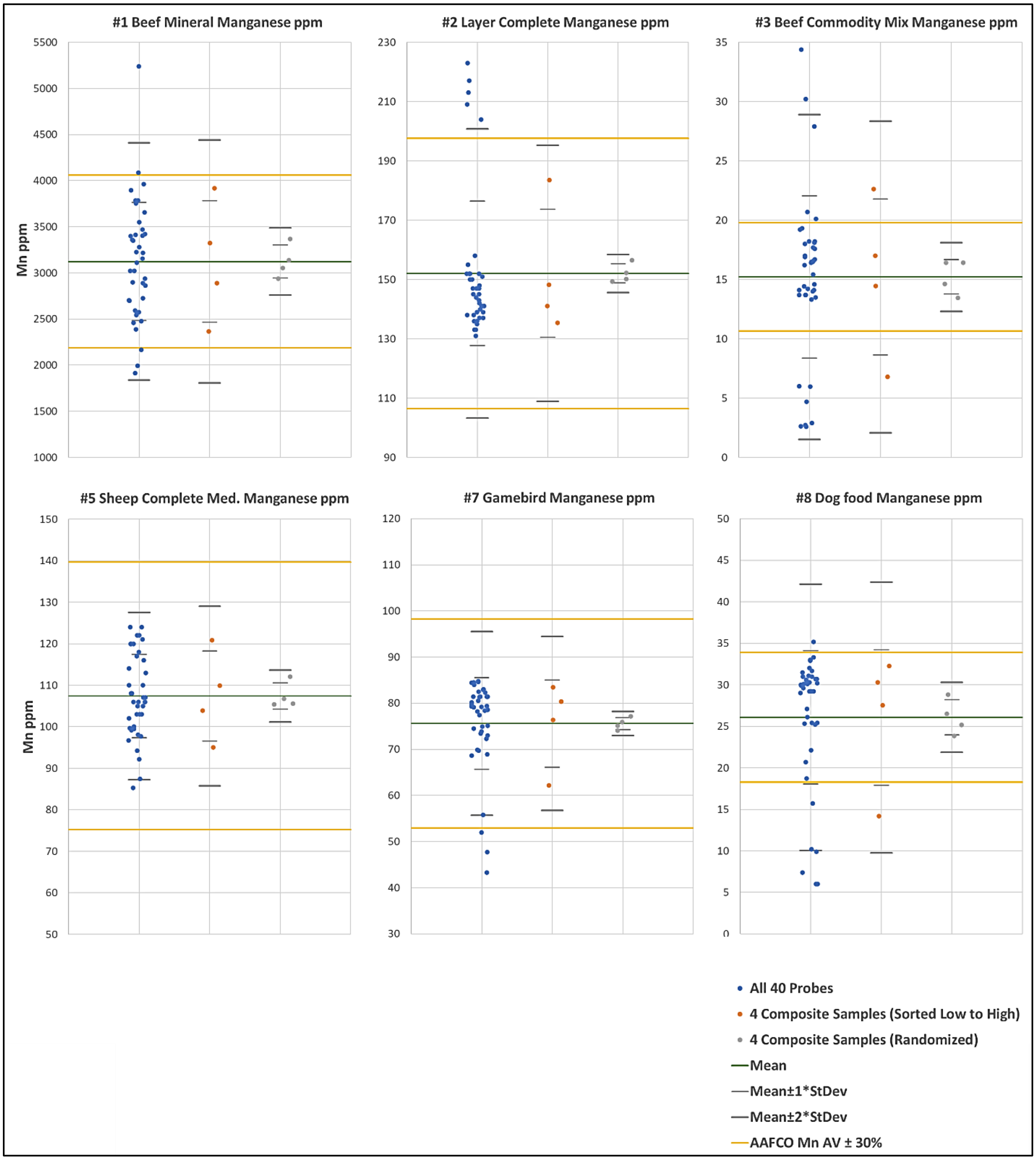

Figure 1i. Manganese results from 40 core samples of six feed types. Data shown for all individual cores, worst-case scenario of 4 composite samples of 10 cores per sample determined from 40 cores ranked from low to high concentration, and a randomized selection of 4 composite samples of 10 cores per sample from the 40 cores. Mean from the 40 individual core results and AAFCO AV of that mean are also shown for comparison.

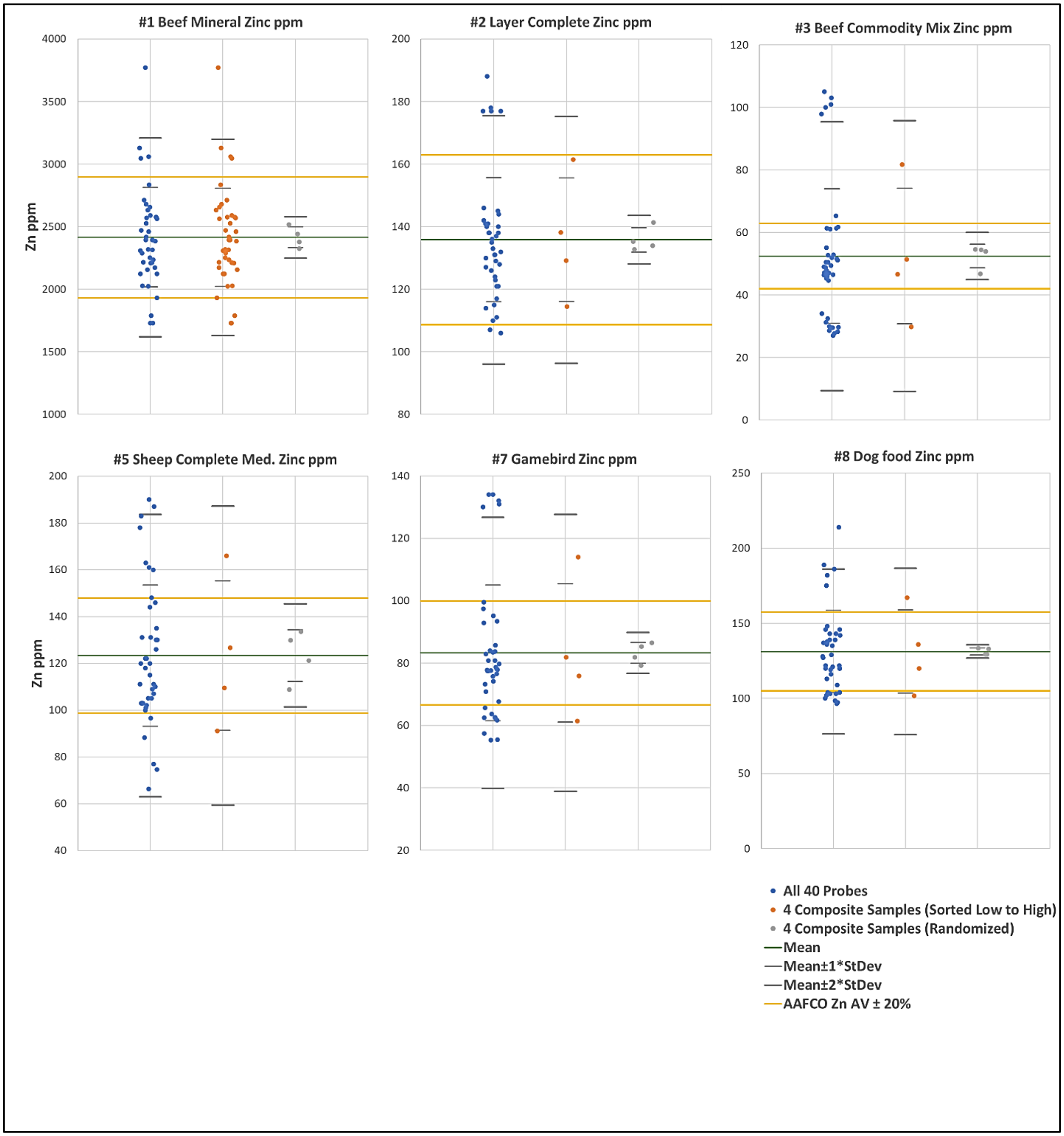

Figure 1j. Zinc results from 40 core samples of six feed types. Data shown for all individual cores, worst-case scenario of 4 composite samples of 10 cores per sample determined from 40 cores ranked from low to high concentration, and a randomized selection of 4 composite samples of 10 cores per sample from the 40 cores. Mean from the 40 individual core results and AAFCO AV of that mean are also shown for comparison.

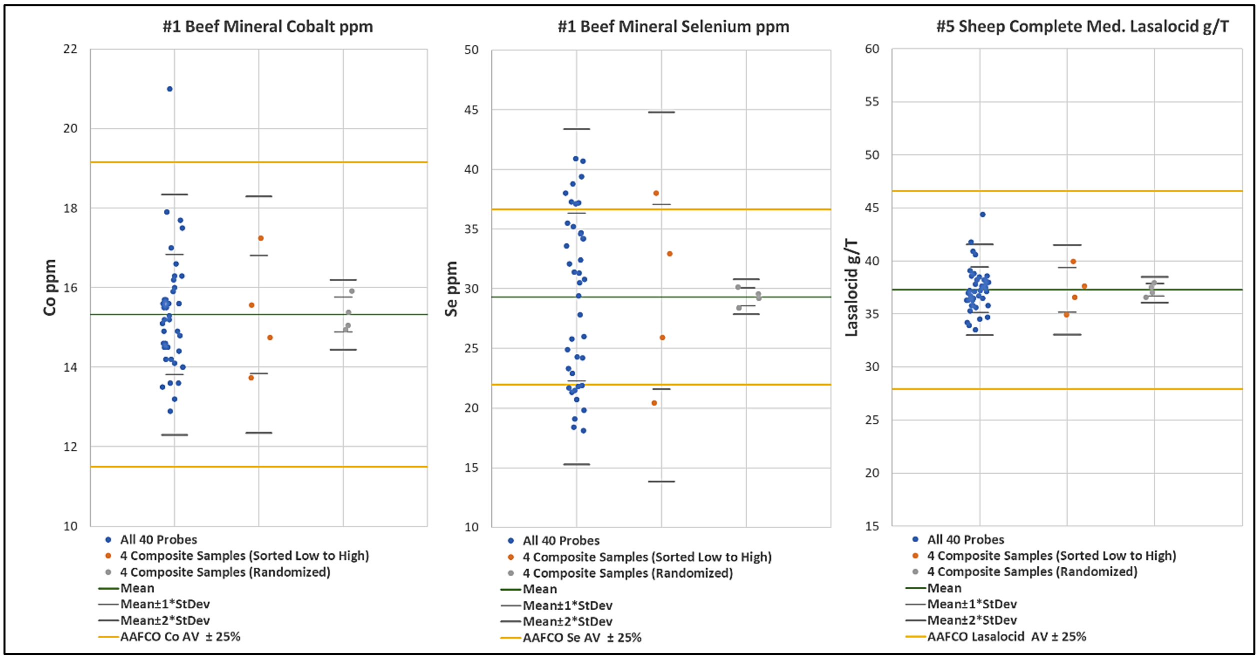

Figure 1k. Cobalt, Selenium, and Lasalocid results from 40 core samples each from a single feed type. Data shown for all individual cores, worst-case scenario of 4 composite samples of 10 cores per sample determined from 40 cores ranked from low to high concentration, and a randomized selection of 4 composite samples of 10 cores per sample from the 40 cores. Mean from the 40 individual core results and AAFCO AV of that mean are also shown for comparison.

Table 4a. Analyte concentrations of a randomized selection of 4 composite samples of 10 cores per sample (4RC) in the beef mineral, the first of eight feed types. Mean and standard deviation of 4 composite samples are shown. Lower and upper limits of 1 standard deviation, 2 standard deviations, and AAFCO AV limits are also shown.

		1 Beef Mineral					
		Mean (4RC)	SD	AAFCO AV ±	4RC mean ± 1 SD Upper Lower	4RC mean ± 2 SD Upper Lower	AAFCO AV limits Upper Lower
Calcium	%	11.36	0.17	10%	11.53 11.19	11.70 11.02	12.49 10.22
Phosphorus	%	3.45	0.07	3/x+8	3.53 3.38	3.60 3.30	3.76 3.15
Magnesium	%	11.32	0.12	20%	11.44 11.20	11.56 11.08	13.58 9.06
Sodium	%	6.90	0.20	15%	7.10 6.70	7.30 6.49	7.93 5.86
Potassium	%	0.28	0.02	15%	0.30 0.27	0.31 0.25	0.32 0.24
Cobalt	ppm	15	0.44	25%	15.76 14.88	16.20 14.44	19.15 11.49
Copper	ppm	1057	25	25%	1082 1031	1108 1006	1321 793
Iron	ppm	4625	69	25%	4694 4556	4762 4488	5781 3469
Manganese	ppm	3124	181	30%	3305 2943	3486 2761	4061 2187
Selenium	ppm	29	0.74	25%	30 29	31 28	37 22
Zinc	ppm	2415	83	20%	2498 2332	2581 2250	2898 1932

Table 4b. Analyte concentrations of a randomized selection of 4 composite samples of 10 cores per sample (4RC) in the layer complete feed, the second of eight feed types. Mean and standard deviation of 4 composite samples are shown. Lower and upper limits of 1 standard deviation, 2 standard deviations, and AAFCO AV limits are also shown.

		2 Layer Complete					
		Mean (4RC)	SD	AAFCO AV ±	4RC mean ± 1 SD Upper Lower	4RC mean ± 2 SD Upper Lower	AAFCO AV limits Upper Lower
Protein	%	21.17	0.04	(20/x+2)	21.22 21.13	21.26 21.09	21.80 20.55
Calcium	%	3.65	0.08	10%	3.73 3.57	3.81 3.49	4.01 3.28
Phosphorus	%	0.84	0.01	3/x+8	0.85 0.83	0.85 0.83	0.94 0.74
Magnesium	%	0.31	<0.01	20%	0.31 0.31	0.31 0.31	0.37 0.25
Sodium	%	0.04	<0.01	15%	0.04 0.04	0.05 0.04	0.05 0.04
Potassium	%	1.18	<0.01	15%	1.19 1.18	1.19 1.17	1.36 1.00
Copper	ppm	13.57	0.79	25%	14 13	15 12	17 10
Iron	ppm	211.8	1.91	25%	214 210	216 208	265 159
Manganese	ppm	152.1	3.20	30%	155 149	159 146	198 106
Zinc	ppm	135.8	3.87	20%	140 132	144 128	163 109

Table 4c. Analyte concentrations of a randomized selection of 4 composite samples of 10 cores per sample (4RC) in the beef commodity mix, the third of eight feed types. Mean and standard deviation of 4 composite samples are shown. Lower and upper limits of 1 standard deviation, 2 standard deviations, and AAFCO AV limits are also shown.

		3 Beef Commodity Mix					
		Mean (4RC)	SD	AAFCO AV ±	4RC mean ± 1 SD Upper / Lower	4RC mean ± 2 SD Upper / Lower	AAFCO AV limits Upper / Lower
Protein	%	11.32	0.29	(20/x+2)	11.61 11.03	11.89 10.75	11.75 10.89
Calcium	%	0.25	0.01	10%	0.27 0.24	0.28 0.22	0.28 0.23
Phosphorus	%	0.49	0.02	3/x+8	0.50 0.47	0.52 0.45	0.55 0.42
Magnesium	%	0.22	0.01	20%	0.23 0.21	0.24 0.20	0.26 0.17
Sodium	%	0.22	0.02	15%	0.23 0.20	0.25 0.19	0.25 0.18
Potassium	%	1.06	0.04	15%	1.10 1.02	1.15 0.98	1.22 0.90
Iron	ppm	205.3	9.79	25%	215 196	225 186	257 154
Manganese	ppm	15.22	1.45	30%	17 14	18 12	20 11
Zinc	ppm	52.46	3.78	20%	56 49	60.0 45	63 42

Table 4d. Analyte concentrations of a randomized selection of 4 composite samples of 10 cores per sample (4RC) in the soybean meal, the fourth of eight feed types. Mean and standard deviation of 4 composite samples are shown. Lower and upper limits of 1 standard deviation, 2 standard deviations, and AAFCO AV limits are also shown.

		4 Soybean Meal					
		Mean (4RC)	SD	AAFCO AV ±	4RC mean ± 1 SD Upper Lower	4RC mean ± 2 SD Upper Lower	AAFCO AV limits Upper Lower
Protein	%	44.15	0.46	(20/x+2)	44.62 43.69	45.08 43.23	45.24 43.07

Table 4e. Analyte concentrations of a randomized selection of 4 composite samples of 10 cores per sample (4RC) in the sheep complete medicated, the fifth of eight feed types. Mean and standard deviation of 4 composite samples are shown. Lower and upper limits of 1 standard deviation, 2 standard deviations, and AAFCO AV limits are also shown.

		5 Sheep Complete BOV					
		Mean (4RC)	SD	AAFCO AV ±	4RC mean ± 1 SD Upper Lower	4RC mean ± 2 SD Upper Lower	AAFCO AV limits Upper Lower
Protein	%	15.92	0.07	(20/x+2)	15.99 15.86	16.06 15.79	16.44 15.41
Calcium	%	0.95	0.01	10%	0.97 0.94	0.98 0.93	1.05 0.86
Phosphorus	%	0.77	0.01	3/x+8	0.78 0.76	0.79 0.75	0.86 0.68
Magnesium	%	0.29	<0.01	20%	0.29 0.29	0.29 0.29	0.35 0.23
Sodium	%	0.33	<0.01	15%	0.34 0.33	0.34 0.32	0.38 0.28
Potassium	%	1.43	0.01	15%	1.43 1.42	1.44 1.41	1.64 1.21
Copper	ppm	8.62	0.67	25%	9.3 8.0	10.0 7.3	10.8 6.5
Iron	ppm	240.75	4.71	25%	246 236	250 231	301 181
Manganese	ppm	107.40	3.14	30%	111 104	114 101	140 75
Zinc	ppm	123.32	11.02	20%	134 112	145 101	148 99
Lasalocid	g/ton	37.27	0.60	25%	38 37	39 36	47 28

Table 4f. Analyte concentrations of a randomized selection of 4 composite samples of 10 cores per sample (4RC) in the distillers dried grains, the sixth of eight feed types. Mean and standard deviation of 4 composite samples are shown. Lower and upper limits of 1 standard deviation, 2 standard deviations, and AAFCO AV limits are also shown.

		6 Distillers Dried Grains					
		Mean (4RC)	SD	AAFCO AV ±	4RC mean ± 1 SD Upper Lower	4RC mean ± 2 SD Upper Lower	AAFCO AV limits Upper Lower
Protein	%	24.34	0.01	(20/x+2)	24.36 24.33	24.37 24.31	25.03 23.65

Table 4g. Analyte concentrations of a randomized selection of 4 composite samples of 10 cores per sample (4RC) in the gamebird feed, the seventh of eight feed types. Mean and standard deviation of 4 composite samples are shown. Lower and upper limits of 1 standard deviation, 2 standard deviations, and AAFCO AV limits are also shown.

		7 Gamebird Feed					
		Mean (4RC)	SD	AAFCO AV ±	4RC mean ± 1 SD Upper Lower	4RC mean ± 2 SD Upper Lower	AAFCO AV limits Upper Lower
Protein	%	18.14	0.07	(20/x+2)	18.20 18.07	18.27 18.01	18.70 17.57
Calcium	%	0.87	0.01	10%	0.88 0.86	0.89 0.84	0.96 0.78
Phosphorus	%	0.59	<0.01	3/x+8	0.59 0.59	0.59 0.59	0.67 0.51
Magnesium	%	0.17	<0.01	20%	0.17 0.17	0.17 0.16	0.20 0.13
Sodium	%	0.16	<0.01	15%	0.16 0.15	0.16 0.15	0.18 0.13
Potassium	%	0.79	0.01	15%	0.80 0.78	0.82 0.76	0.91 0.67
Copper	ppm	11.74	0.78	25%	13 11	13 10	15 9
Iron	ppm	124.91	1.58	25%	127 123	128 122	156 94
Manganese	ppm	75.57	1.31	30%	77 74	78 73	98 53
Zinc	ppm	83.26	3.31	20%	87 80	90 77	100 67

Table 4h. Analyte concentrations of a randomized selection of 4 composite samples of 10 cores per sample (4RC) in the dog food, the last of the eight feed types. Mean and standard deviation of 4 composite samples are shown. Lower and upper limits of 1 standard deviation, 2 standard deviations, and AAFCO AV limits are also shown.

		8 Dog Food					
		Mean (4RC)	SD	AAFCO AV ±	4RC mean ± 1 SD Upper Lower	4RC mean ± 2 SD Upper Lower	AAFCO AV limits Upper Lower
Protein	%	23.53	0.06	(20/x+2)	23.59 23.46	23.65 23.40	24.20 22.86
Calcium	%	1.22	0.03	10%	1.26 1.19	1.29 1.16	1.35 1.10
Phosphorus	%	0.84	0.01	3/x+8	0.85 0.82	0.86 0.81	0.93 0.74
Magnesium	%	0.12	0.01	20%	0.13 0.11	0.14 0.10	0.14 0.10
Sodium	%	0.45	0.01	15%	0.46 0.45	0.47 0.44	0.52 0.39
Potassium	%	0.67	0.01	15%	0.68 0.66	0.69 0.65	0.77 0.57
Copper	ppm	14.64	1.13	25%	16 14	17 12	18 11
Iron	ppm	242.9	4.30	25%	247 239	252 234	304 182
Manganese	ppm	26.08	2.11	30%	28 24	30 22	34 18
Zinc	ppm	131.2	2.25	20%	134 129	136 127	158 105

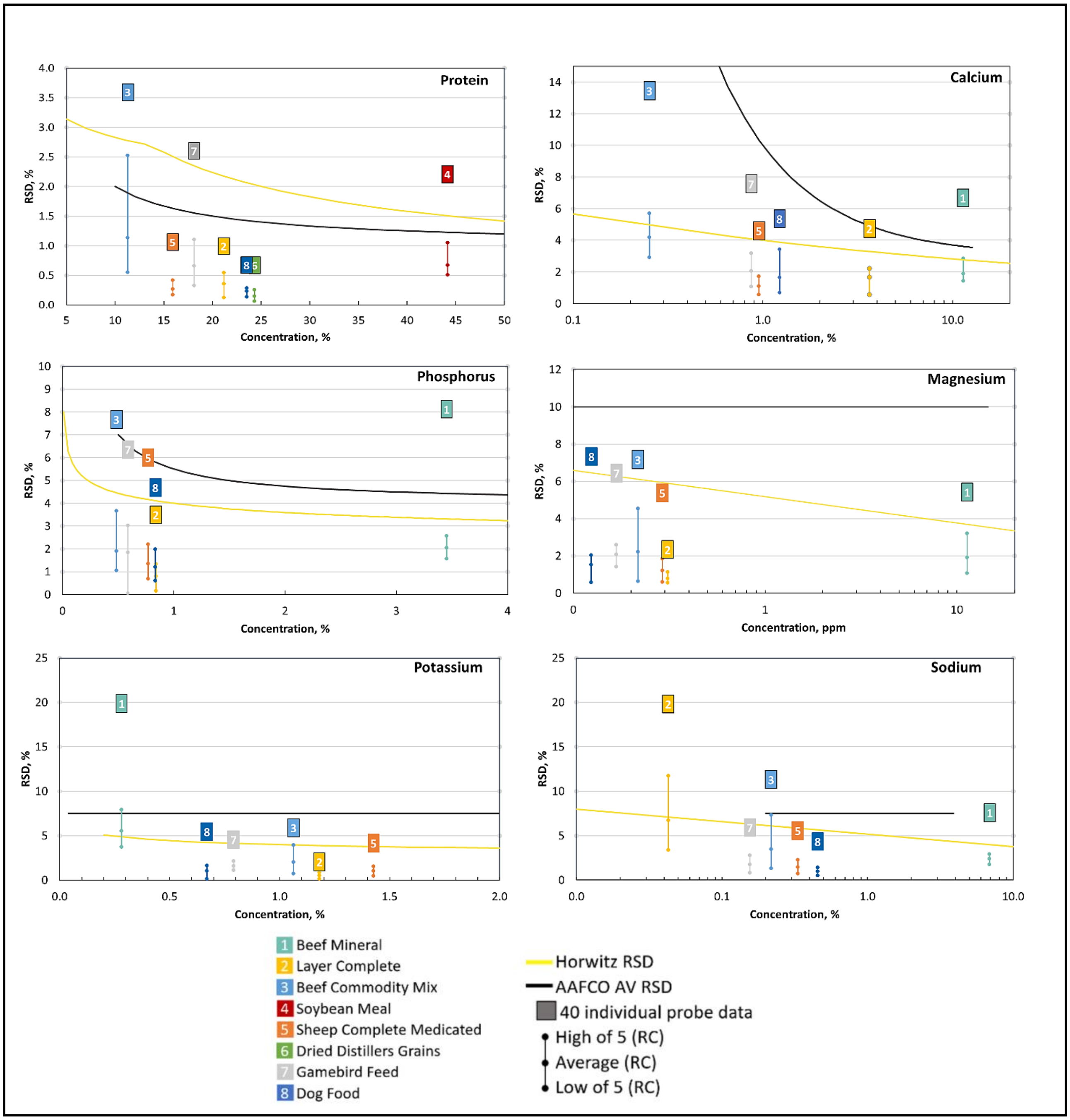

Figure 2a: Global RSD versus concentration for 6 analytes in 8 feed types. Square data points with sample IDs represent the RSD from results in 40 individual cores. Vertical lines below these data points show results for the feed type RSDs from 5 sets of 4 randomized composite samples of 10 cores per sample. The RSD from AAFCO AVs and Horwitz are also shown.

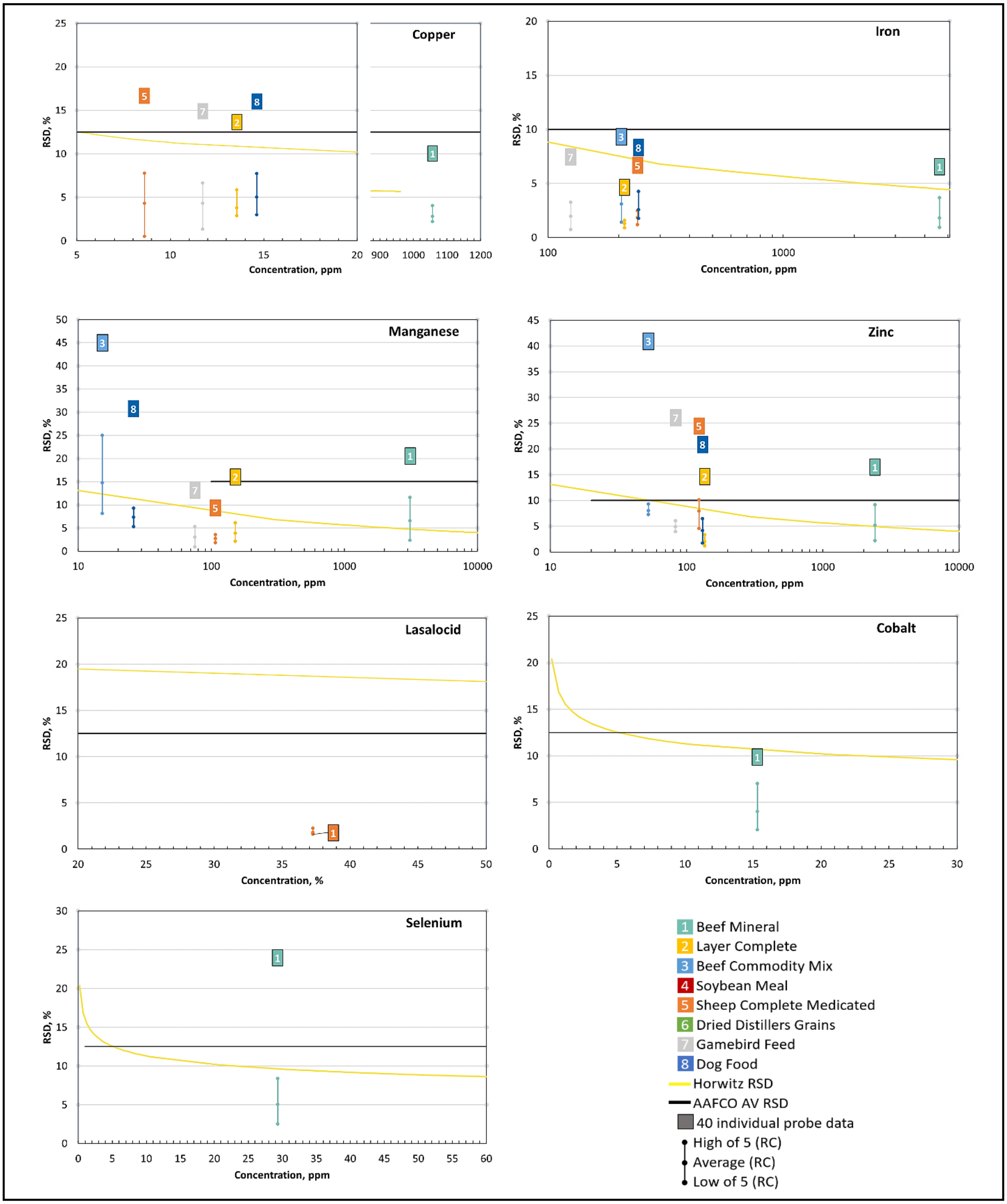

Figure 2b: Global RSD versus concentration for 7 analytes in 8 feed types. Square data points with sample IDs represent the RSD from results in 40 individual cores. Vertical lines below these data

points show results for the feed type RSDs from 5 sets of 4 randomized composite samples of 10 cores per sample. The RSD from AAFCO AVs and Horwitz are also shown.