Effects of Marker Selection and Mix Time on the Coefficient of Variation (Mix Uniformity) of Broiler Feed

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Primary Audience: Feed Mill Managers, Feed Mill Quality Control Managers, Nutritionists

SUMMARY
An experiment was conducted evaluating several markers to determine mix uniformity. Treatment diet was a corn-soybean meal-based diet formulated for broiler chicks fed from 0 to 17 d posthatch. Dietary nutrients or tracers evaluated included the following: 1) DL-Met (99%), 2) L-Lys-HCl (78%), 3) CP, 4) mixing salt (chloride ion), 5) P, 6) Mn, 7) Fe particles (#40 Red, count), 8) Fe particles (#40 Red, absorbance), 9) Fe particles (RF-Blue Lake), 10) roxarsone, and 11) semduramicin. All minor and microingredients were individually hand-weighed and added to the mixer to insure accuracy and were added at the same location for all treatments. Diets were mixed using a double ribbon mixer for 3 different mix times (0.5, 2.5, and 5.0 min). Overall, from 0.5 to 5.0 min, all markers evaluated showed a numerical reduction in percentage of CV. Crude protein and P should not be considered as markers, because many different components in the batch of feed contribute some level of CP or P, and results can be confounding. DL-Methionine (99%) and L-Lys-HCl (78%) were the only markers that statistically reduced over time and had a CV < 10% (23.86 to 9.47% and 19.75 to 8.70%, respectively). These data suggest that mixer uniformity results can be influenced by the particular marker that is chosen for mixer uniformity analysis.

Key words: mixer uniformity, marker selection, coefficient of variation

DESCRIPTION OF PROBLEM
Nutrient uniformity is critical for proper nutrition when feed is being consumed by animals with low daily feed intake (baby chicks, nursery pigs, etc.) [1]. Beumer [2] indicated mix uniformity as one of the critical quality control points in feed manufacturing. Concerns for creating a uniform mix would include the following: 1) nutritional overfortification by the nutritionist [3], 2) regulatory aspects [4], and 3) animal performance [5]. Wicker and Poole [3] reported mixing a 6-ton batch of feed in a typical 5-ton mixer and were unable to reduce the CV below 29.8%, even as mix time was increased. Once the batch size was reduced to 5 tons in the same mixer, mix uniformity (CV) improved dramatically from 34.6 to 2.6% and 12.0 to 4.6% as

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1Mention of trade names or commercial products in this publication is solely to provide specific information and does not imply recommendation or endorsement by Kansas State University.
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mix time was increased (supplemental Met and Lys as markers, respectively). The *Feed Additive Compendium* [4] has the following caution statement on several feed additives: “Must be thoroughly mixed in feeds before use.” This statement is not only for the regulatory aspect in showing a Food and Drug Administration official that your mixer is performing adequately, but one must be able to report assay results for feed additives and other nutrients to guarantee concentration levels stated on feed labels. To accomplish this, a CV < 10% has been used throughout the feed industry as the level that mixer uniformity must achieve to be considered a good mix.

McCoy et al. [5] evaluated broiler performance as affected by mix uniformity and reported an increase in average daily gain (23.6 to 30.6 g), average daily feed intake (43.1 to 52.7 g), and gain:feed (0.548 to 0.576) during the growing phase as mixer revolution (mix time) was increased. In addition, McCoy et al. [5] reported a reduction of mortality from 12.0 to 0.0% as mixer revolution increased.

In assaying a mixer, samples collected will contain a defined marker. In selecting a marker to test for mix uniformity, one should evaluate several characteristics, such as accuracy of the laboratory assay, ease of the assay, assay cost, on-site analysis, common ingredient, and a single source. Pfost et al. [6] stated criteria for marker selection included the following: 1) do not select markers in which variation will not affect animal performance (e.g., vitamin A); 2) select ingredients with similar physical properties (particle size and density); 3) do not utilize characteristics in which almost or all ingredients carry (e.g., ash); 4) analytical assay variability must be less than mixer variability; 5) feed additives (drugs) can make good tracers, because degree of mixing is important from a legal and animal performance standpoint; and 6) mineral elements can be good tracers because of density and particle size; however, high assay costs may be prohibitive.

Several markers are commonly used today in the feed industry ranging from dietary nutrients (DL-Met) to feed additives (semduramicin). Markers can provide forewarning of possible problems taking place in the mixer, such as the following: irregularity of ingredient particle size

**Table 1. Composition of experimental broiler starter diet (as-fed basis)**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>% as-is</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>60.48</td>
</tr>
<tr>
<td>Soybean meal (48.5% CP)</td>
<td>31.55</td>
</tr>
<tr>
<td>Meat meal (porcine, 50% CP)</td>
<td>3.50</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>1.35</td>
</tr>
<tr>
<td>Monocalcium phosphate</td>
<td>1.20</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>0.95</td>
</tr>
<tr>
<td>Salt</td>
<td>0.34</td>
</tr>
<tr>
<td>DL-Met (99%)</td>
<td>0.25</td>
</tr>
<tr>
<td>L-Lys-HCl (78%)</td>
<td>0.03</td>
</tr>
<tr>
<td>Vitamin-mineral premix</td>
<td>0.25</td>
</tr>
<tr>
<td>3-Nitro 202</td>
<td>0.05</td>
</tr>
<tr>
<td>Aviax 5%3</td>
<td>0.05</td>
</tr>
<tr>
<td>Marker4 (mg/kg)</td>
<td>55.06</td>
</tr>
<tr>
<td>Marker5 (mg/kg)</td>
<td>55.06</td>
</tr>
<tr>
<td><strong>Calculated composition</strong></td>
<td></td>
</tr>
<tr>
<td>ME, kcal/kg</td>
<td>3,058.00</td>
</tr>
<tr>
<td>CP, %</td>
<td>22.20</td>
</tr>
<tr>
<td>Lys, %</td>
<td>1.23</td>
</tr>
<tr>
<td>Met + cystine, %</td>
<td>0.96</td>
</tr>
<tr>
<td>Ca, %</td>
<td>0.95</td>
</tr>
<tr>
<td>Total P, %</td>
<td>0.70</td>
</tr>
<tr>
<td><strong>Analyzed composition, %</strong></td>
<td></td>
</tr>
<tr>
<td>CP</td>
<td>21.00</td>
</tr>
<tr>
<td>L-Lys-HCl</td>
<td>0.025</td>
</tr>
<tr>
<td>DL-Met</td>
<td>0.243</td>
</tr>
<tr>
<td>Ca</td>
<td>0.96</td>
</tr>
<tr>
<td>Total P</td>
<td>0.75</td>
</tr>
</tbody>
</table>

1Provided the following (per kg of complete diet): 7,717 IU of vitamin A, 1,653 IU of vitamin D, 16 IU of vitamin E, 0.83 mg of vitamin K, 385 mg of biotin, 24 mg of Cu, 3.3 mg of I, 110 mg of Fe, 220 mg of Mn, 27 mg of niacin, 6 mg of pantothenic acid, 6 mg of riboflavin, 0.3 mg of Se, and 1 mg of thiamin.

2Provided 50 mg of Roxarsone per kilogram of complete diet.

3Provided 25 mg of semduramicin per kilogram of complete diet.

4Microtracer Red #40 Fe marker added at 50 g/ton.

5Microtracer RF-Blue Lake Fe marker added at 50 g/ton.

[7], mixer buildup [8], worn or broken parts [8], over- or underfilling [3, 8], ingredient sequencing [9], improper mixer adjustment [10], or inadequate mix time [11]. Numerous markers are being utilized for determining mix uniformity. The objective of this trial was to evaluate the effects of marker selection and mix time on CV in the mixing process.

**MATERIALS AND METHODS**

A corn-soybean meal-based diet was formulated for broiler chickens during the starter phase (0 to 17 d; Table 1). For the evaluation of mix
Table 2. Experimental mix times for determination of mixer CV

<table>
<thead>
<tr>
<th>Mix level</th>
<th>Dry mix (s)</th>
<th>Wet mix (s)</th>
<th>Total mix time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>0</td>
<td>30</td>
<td>0.5</td>
</tr>
<tr>
<td>Medium</td>
<td>60</td>
<td>90</td>
<td>2.5</td>
</tr>
<tr>
<td>High</td>
<td>120</td>
<td>180</td>
<td>5.0</td>
</tr>
</tbody>
</table>

uniformity, dietary nutrients or tracers in the feed that were analyzed included the following: 1) DL-Met (99%), 2) L-Lys-HCl (78%), 3) CP, 4) mixing salt (chloride ion), 5) P, 6) Mn, 7) Fe particles ([12] #40 Red count [MTC]), 8) Fe particles ([12] #40 Red absorbance [MTA]), 9) Fe particles ([12] RF-Blue Lake [MTB]), 10) roxarsone, and 11) semduramicin.

Coefficient of variation was calculated by:

\[
\%CV = \frac{s}{m} \times 100
\]

\[
m = \frac{\sum x_i}{n}
\]

\[
s^2 = \frac{\sum(x_i^2) - nm^2}{n - 1},
\]

where \(\%CV\) = percentage of CV; \(s\) = standard deviation; \(s^2\) = variance; \(m\) = mean; \(x_i\) = individual sample analysis results; and \(n\) = number of samples assayed.

Feed Manufacturing

Diets were mixed using a double-ribbon mixer (454-kg capacity) [13]. The mixer was physically cleaned so that no residual build-up was present on the ribbons or the mixer wall before mixing to reduce confounding effects of mixer build-up on final CV. Diets were batched as to utilize full capacity of the mixer on a weight basis (454 kg/batch). Soybean oil (1%) was added to all batches at the mixer. Mix times (Table 2) included both a dry mix time and wet mix time. Dry mix time began after all dry ingredients were added in an idle mixer at the instant the mixer began to turn. At the completion of the dry mix cycle, the wet mix cycle immediately began and continued while soybean oil was being applied into the mixer. Mix time 0.5 min was required to add the soybean oil into the batch, whereas 2.5 min was 50% of the Kansas State University Feed Processing Centers Standard Operating Procedures for mixing of 120-s dry mix and 180-s wet mix times (5 min total). All minor and microingredients were individually hand weighed and added to the mixer at the same location to insure accuracy of inclusion rates and consistency.

After the required mix time, the mixer was stopped, and the discharge gate was opened. Mixed feed was conveyed by a screw conveyor to a bucket elevator, elevated 21.34 m (70 ft), and discharged through a gravity spout 16.76 m (55 ft), passing through a turn-head to a packaging bin where the mash was packaged. Mixed feed was packaged continuously to reduce potential further mixing in the bin upon discharge. Mash samples (5 kg) were collected from sacks (\(n = 10\); i.e., 1, 3, 5, etc.) using a multiport sample probe [14] and then divided, using a sample splitter, into appropriate aliquots for laboratory analysis. Within each sample, all markers were analyzed for that batch. Laboratory analysis procedures used for each nutrient or marker are summarized in Table 3.

Statistics

Data were analyzed as a completely randomized design. Mixer batch served as the experimental unit for all analyses (replication, \(n = 3\)). All data were analyzed using the MIXED procedure of SAS [15] by orthogonal contrasts evaluating linear and quadratic responses in addition to pairwise comparison between mix times within marker. Statistical significance was established at \(P < 0.05\).

RESULTS AND DISCUSSION

Results for mix uniformity, as determined by CV over time, are reported in Table 4. All marker data resulted in a numerical reduction in CV from 0.5 to 2.5 min of mix time, ranging from 5.7 (CP) to 52.9% (P); however, there were no statistical differences detected within any marker from 0.5 to 2.5 min. From 2.5 to 5.0 min, CV for salt and roxarsone increased slightly numerically, whereas all other markers continued to reduce in CV. Overall, from 0.5 to 5.0 min, all markers showed a numerical reduction in CV. These data are in agreement with McCoy et al. [5], who evaluated salt (chloride and sodium), MTC, MTB, and Cr and reported a reduction in CV during the first period (−25%) of the
Table 3. Laboratory analysis utilized for marker detection.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Method of analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>DL-Met</td>
<td>AOAC 994.12 [23]</td>
</tr>
<tr>
<td>Lys-HCl</td>
<td>AOAC 994.12 [23]</td>
</tr>
<tr>
<td>CP</td>
<td>AOAC 990.03 [23]</td>
</tr>
<tr>
<td>Chloride ion (as sodium chloride)</td>
<td>[18]</td>
</tr>
<tr>
<td>P</td>
<td>[19]</td>
</tr>
<tr>
<td>Mn</td>
<td>AOAC 968.08 [23]</td>
</tr>
<tr>
<td>Microtracer Red #40 (count)</td>
<td>[20]</td>
</tr>
<tr>
<td>Microtracer Red #40 (absorbance)</td>
<td>[21]</td>
</tr>
<tr>
<td>Microtracer RF-Blue Lake</td>
<td>[22]</td>
</tr>
<tr>
<td>Roxarsone (3-Nitro)</td>
<td>AOAC 971.47 [23]</td>
</tr>
<tr>
<td>Semduramicin (Aviax)</td>
<td>Phibro S188.21</td>
</tr>
</tbody>
</table>

1Assay and Identity of Semduramicin in Feeds by Normal-Phase Liquid Chromatography with Postcolumn Reaction.

The rapid numerical decrease in CV during the first period of mixing, as reported from this experiment, is also in agreement with Wilcox and Unruh [8], who tested several models of mixers, evaluating CV. Previous research [16] investigated the dispersion of sodium chloride and nitrophenide simultaneously in a series of experiments with these markers incorporated into the following: 1) soybean meal, 2) corn ground through a 1/8-in. screen (1/8-in. corn), 3) corn ground through a 6/64-in. screen (6/64-in. corn), and 4) complete poultry diet. The author reported findings that suggested reducing particle size of the carrier (soybean meal, 1/8-in. corn, 6/64-in. corn, or complete poultry diet) had a significant effect on mix uniformity. Differences in particle size and density of the marker also had an effect on the final distribution of the material. Not only did the physical characteristics of the markers and the mash result in different responses on mix uniformity, but neither marker followed similar trends for estimating mix uniformity. This would be in agreement with McCoy et al. [5], who tested 4 different markers simultaneously at an intermediate mix time and reported results that ranged from 12.1 to 23.2%, depending upon which method was chosen for CV determination.

The current experiment was designed for a practical application. Inclusion levels of the microingredients, nutrient concentrations, or both, are comparable to what is currently practiced in industry. All markers evaluated were not included in the diet at the same level or measured using the same analytical techniques. This is in contrast to a nutritionist, for example, comparing the effects of 3 protein sources on animal perfor-

Table 4. Effect of marker selection and mix time on CV in the mixing process

<table>
<thead>
<tr>
<th>Marker, %CV</th>
<th>Mix time (min)</th>
<th>0.5</th>
<th>2.5</th>
<th>5.0</th>
<th>Linear</th>
<th>Quadratic</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>DL-Met (99%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>23.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.56&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.644</td>
<td>0.241</td>
<td>7.43</td>
</tr>
<tr>
<td>L-Lys-HCl (78%)</td>
<td></td>
<td>19.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.021</td>
<td>0.011</td>
<td>1.66</td>
</tr>
<tr>
<td>CP</td>
<td></td>
<td>7.73</td>
<td>7.29</td>
<td>6.86</td>
<td>0.828</td>
<td>0.702</td>
<td>1.33</td>
</tr>
<tr>
<td>Chloride ion (as sodium chloride)</td>
<td></td>
<td>20.26</td>
<td>12.75</td>
<td>15.08</td>
<td>0.664</td>
<td>0.201</td>
<td>3.60</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>13.72</td>
<td>6.46</td>
<td>6.27</td>
<td>0.951</td>
<td>0.027</td>
<td>2.07</td>
</tr>
<tr>
<td>Mn</td>
<td></td>
<td>36.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.59&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.535</td>
<td>0.007</td>
<td>3.45</td>
</tr>
<tr>
<td>Microtracer Red #40 (count)</td>
<td></td>
<td>21.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.72&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.619</td>
<td>0.002</td>
<td>1.73</td>
</tr>
<tr>
<td>Microtracer Red #40 (absorbance)</td>
<td></td>
<td>21.13</td>
<td>20.52</td>
<td>16.88</td>
<td>0.592</td>
<td>0.929</td>
<td>5.13</td>
</tr>
<tr>
<td>Microtracer RF-Blue Lake</td>
<td></td>
<td>32.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.772</td>
<td>0.019</td>
<td>3.36</td>
</tr>
<tr>
<td>Roxarsone (3-Nitro)</td>
<td></td>
<td>30.42</td>
<td>25.15</td>
<td>25.54</td>
<td>0.965</td>
<td>0.511</td>
<td>5.94</td>
</tr>
<tr>
<td>Semduramicin (Aviax)</td>
<td></td>
<td>27.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.436</td>
<td>0.035</td>
<td>4.13</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>Different letters within rows represent statistical significance (P < 0.05).

<sup>1</sup>Probability of statistical significance (P < 0.05).
performance and formulating the diets to be isocaloric and isonitrogenous so that conclusions could be made about the protein source (i.e., digestibility).

Crude protein should not be considered for use as a marker because all major components contribute some level of protein (in addition to certain minor or microingredients), and it can be difficult to determine if the batch has been mixed adequately or not. At the initial mix time, protein had a CV of <10%, and the mixer had only operated for 30 s. Once again, in practice, feed manufacturers would never utilize a mix time of 30 s. To illustrate why CP is an inferior choice for a marker, assume a 2-ton mixer is filled with 1 ton of soybean meal (48% CP) and 1 ton of porcine meat meal (50% CP). If the mixer was not turned on but 10 samples were taken for mixer analysis (and we assume no sampling error and exact nutrient profiles), 2.15% CV is the highest the mixer CV could be. Phosphorus has a similar response of CP in that it started with a relatively low CV initially (13.72%) and reduced to only 6.46 and 6.27% CV for 2.5 and 5.0 min of mix time, respectively. Although there was a quadratic response (P < 0.027) for P, no statistical differences were found using P or CP as a marker for the 3 mix times.

Salt (chloride ion) followed similar trends as McCoy et al. [5], with a decrease from the initial mix time to the intermediate mix time but then a slight increase from the intermediate to the high mix time. One would assume salt as a single source; however, analytical tests were performed measuring the chloride ion concentration. L-Lysine-HCl, which was included in the diet, does contain the chloride ion and has the potential to distort the results. Choline chloride was not included in this experimental diet, but it is readily used in the industry and should be considered if a chloride analysis is used.

Manganese showed a reduction in CV from 0.5 to 2.5 min (36.25 to 20.80%) and, during the last period (2.5 to 5.0 min), a reduced CV (20.80 to 17.59 %, respectively) resulting in an overall (0.5 to 5.0 min) curvilinear decrease (quadratic P < 0.007).

All Fe markers (MTC, MTA, and MTB) resulted in an overall numerical reduction (0.5 to 5.0 min) in CV (21.77 to 10.43%, 21.13 to 16.88%, and 32.49 to 18.64%, respectively). For both MTC and MTB a quadratic decrease (P < 0.002 and P < 0.019, respectively) was shown, and a statistical decrease from 2.5 to 5.0 min was also observed for both MTC and MTB. Although MTC and MTA are the same ingredient, the analytical techniques used are different. The analytical assay for MTC is reported by counting particles within a set area, whereas MTA uses a spectrophotometer and reads absorbance. Interference from other ingredients within the batch could contribute to some of the differences that were noticed between MTC and MTA. Other opportunities that could be considered include the use of Fe particle products [12] to identify potential carryover within the batching system.

Roxarsone had a minimal reduction in CV overall (30.42 to 25.54%), whereas semduramicin resulted in a CV reduction of 27.40 to 11.23% from 0.5 to 5.0 min, respectively. Even though both feed additives had the same inclusion level and were placed in the mixer at the same location, analytical variability (AV) may have been a factor. Analytical variability is allowed [17] for both feed additives; however, roxarsone has a greater allowable AV (85 to 120%) compared with semduramicin (80 to 110%), and this AV could explain differences in final CV results.

Finally, the CV for both supplemented DL-Met and L-Lys-HCl decreased over time (0.5 to 5.0 min) from 23.86 and 19.75% to 9.47 and 8.70%, respectively. A linear (P < 0.021) and quadratic (P < 0.011) response was observed for Lys, whereas no linear or quadratic differences (P < 0.644 or P < 0.241, respectively) were observed for DL-Met. However, in evaluating pairwise differences within the marker, there were significant differences detected for both DL-Met and L-Lys-HCl from 0.5 to 5.0 min.

CONCLUSIONS AND APPLICATIONS

1. Crude protein and P are not desirable markers, because there are many ingredients that are potential sources and that could confound the results.
2. Feed additive (antibiotics, etc.) assays can be cost-prohibitive, and analytical variability can be unfavorable, with many AV allowances ranging from 80 to 120% of the target.
3. Manganese, MTC, MTB, and semduramicin did show a decrease statistically; however, they did not fall below a target CV of 10%.
4. Supplemental DL-Met (99%) and L-Lys-HCl (78%) were the only markers that statistically reduced over time and had a CV < 10% after 5.0 min of mixing.
5. Each mixer analysis will be unique due to diet formulation, particle size of the raw ingredients, and measure on the mixer parts, mixer cleanliness, individual sampling, mixing time, and the marker chosen for mixer uniformity.

REFERENCES AND NOTES

13. Sprout Waldron, Muncy, PA.
15. Release 9.1 for Windows, SAS Institute, Cary, NC.
18. Quantab Chloride Titrator Procedure (Quantab, Hach Co., Loveland, CO)
   1) Weigh 10 g of sample into a cup.
   2) Add 90 mL of boiling distilled water, stir for 30 s, wait 60 s, and stir another 30 s.
   3) Fold a circle of filter paper in half twice, and open the cone-shaped cup and place the filter into the solution. Allow liquid to permeate filter paper.
   4) Place the titrator in the solution with the filter cone. Do not puncture a hole into the filter paper. The titrator capillary tube may become plugged with small feed particles.
   5) Leave the titrator strip in the solution until the yellow indicator strip across the top has turned dark blue.
   6) Remove the titrator, and record the reading to the nearest one-half division on the numbered scale.
   7) Convert the reading to percentage of salt using the calibration tables provided with the titrators.
   8) Multiply the percentage of salt from the table by 10 to adjust for dilution.
   9) Calculate the CV for each set of samples analyzed.
19. Phosphorus Determination in Biological Material

Reagents

MS Solution: Dissolve 5 g of sodium molybdate (Na2MoO4 + 2H2O) in 500 mL of water. Add 14 mL of 36% H2SO4 and bring to 1 L with water.

Elon Solution: Dissolve 1 g of elon (p-methylaminophenol sulfonyl) in 100 mL of 3% sodium bisulfate (NaHSO3). Store under refrigeration.

Standards: From potassium dihydrogen phosphate (KH2PO4), prepare standards containing 20, 40, 60, 100, and 200 μg of P/mL.

Sample Preparation

Weigh 2 g into acid-washed crucibles. Ash at 600°C, increasing temperature slowly. Dissolve in 5 mL of 6 N HCl, bring to a boil, cool, and bring to 100 mL with deionized distilled H2O. Add 0.3 mL to MS solution and read against standards prepared by diluting 0.3 mL of P standards containing 50, 100, and 200 μg of P/mL with MS solution.

Procedure

Dilute a sample volume containing 30 μg of P with 4 mL of MS solution. Mix, and add 0.5 mL of elon solution. Mix again, and let stand for 1 h. Read at 700 nm on spectrophotometer.

20. Microtracer Rotary Detector Procedure (Red Count)
   1) Remove upper hopper.
   2) Place a 7-cm circle of #1 Whatman filter paper, with a hole in the center, on the rotary magnet.
   3) Replace upper hopper.
   4) Turn the rotary detector on.
   5) Slowly pour a 25-g sample through the upper hopper. Run the sample through the rotary detector a second time to increase particle recovery. Turn off rotary detector.
   6) Remove the upper hopper and carefully remove the filter paper from the rotary magnet.
   7) Transfer particles from the filter paper to a brass or aluminum scoop. Brush the filter paper if necessary.
   8) Slowly pass a bulk tape demagnetizer under the scoop, gradually moving it downward. This demagnetizes the particles and causes them to appear individually.
   9) Transfer the particles from the scoop to a circle of #1 Whatman filter paper (11 cm or larger), spreading them as uniformly as possible.
   10) Place the piece of filter paper with evenly distributed particles on a pie pan and thoroughly wet with 70% ethanol.

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11) Place the pie pan on a preheated hot plate to dry the filter paper.
12) After the filter paper has dried, brush it clean and count the number of spots of each color.
13) Repeat the process the additional time, and determine the particle count for a 100-g sample.
14) Calculate the CV for each set of samples tested.

21. Microtracers Red #40 Absorbance Procedure

**Equipment**
1. Spectrophotometer with 1-cm cell.
2. Rotary detector with rare earth magnet.
3. 50-mL screw-cap culture tubes.
4. 20-mL or 10-mL calibrated pipette.
5. Balance suitable for weighing 100 g with gradient of 0.1 g or better.

**Reagents**
1. Microtracer RF-Blue Lake.
2. Deionized water.

**Tracer Addition**
Microtracer RF-Blue Lake should be added at 50 ppm (i.e., 50 g of tracer per metric ton of final feed). This tracer should be premixed in 250 g of carrier (i.e., ground corn, salt, etc.) before adding the tracer to the mix.

**Assay**
Mix 50 mg of RF-Blue #1 Lake (lot A-5965) with 1,000 g of mash feed sample in a small mixer for 20 min.
1) Take a 75-g subsample, and pass the subsample twice through the rotary detector with rare earth magnet to retrieve Fe particles. Recovery of the tracer will be 99%+. 2) Transfer all the retrieved Fe particles to a 30-mL weigh scoop. Place the scoop on a demagnetizer, sliding it while blowing across it gently to free retained dust as much as reasonably possible.
3) Transfer the retrieved Fe to a 50-mL screw-cap culture tube, and add 20.00 mL of deionized water by a calibrated pipette. Agitate the tube by shaking vigorously for 20 min to release and disperse the dye.
4) If the solution is turbid, filter the solution using slow or medium qualitative filter paper.
5) Read the absorbance at 630 nm after establishing a blank on the spectrophotometer with deionized water.

22. Microtracers RF-Blue #1 Procedure

**Equipment**
1. Spectrophotometer with 1-cm cell.
2. Rotary detector with rare earth magnet.
3. 50-mL screw-cap culture tubes.
4. 20-mL or 10-mL calibrated pipette.
5. Balance suitable for weighing 100 g with gradient of 0.1 g or better.

**Reagents**
1. Microtracer RF-Blue Lake.
2. 1% sodium carbonate aqueous solution.

**Tracer Addition**
Microtracer RF-Blue Lake should be added at 50 ppm (i.e., 50 g of tracer per metric ton of final feed). This tracer should be premixed in 250 g of carrier (i.e., ground corn, salt, etc.) before adding the tracer to the mix.

**Assay**
Mix 50 mg of RF-Blue #1 Lake (lot A-5965) with 1,000 g of mash feed sample in a small mixer for 20 min.
1) Take a 75-g subsample, and pass the subsample twice through the rotary detector with rare earth magnet to retrieve Fe particles. Recovery of the tracer will be 99%+.
2) Transfer all the retrieved Fe particles to a 30-mL weigh scoop. Place the scoop on a demagnetizer, sliding it while blowing across it gently to free retained dust as much as reasonably possible.
3) Transfer the retrieved Fe to a 50-mL screw-cap culture tube, and add 20.00 mL of 1% sodium carbonate aqueous solution by a calibrated pipette. Agitate the tube by shaking vigorously for 20 min to release and disperse the dye.
4) If the solution is turbid, filter the solution using slow or medium qualitative filter paper.
5) Read the absorbance at 630 nm after establishing a blank on the spectrophotometer with 1% sodium carbonate aqueous solution.


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