ORIGINAL RESEARCH

Mixing and clean-out properties of sulfamethazine and carbadox in swine feed

Timothy J. Herrman, PhD; Keith C. Behnke, PhD; and Thomas M. Loughin, PhD

Summary: *Two forms of sulfamethazine (pelleted and granular) and one form of carbadox (granular) were evaluated for their mixing and clean-out properties in replicated batches of swine feed prepared at the research feed mill at Kansas State University. Analysis of variance indicated that carbadox was incorporated into the feed more uniformly than sulfamethazine. Additional mixing beyond 1.5 minutes did not improve drug distribution (P>0.05). A paired-comparison T-test was used to compare mixing properties of drugs versus salt (the latter is commonly used to test mixer performance). We found the mixing properties of sulfamethazine and salt to be different (P < 0.05), whereas those of carbadox and salt did not differ (P > 0.05). Ground corn was used to flush the mixer and conveying system between feed batches; then the mixer, leg, and sack-off bin were cleaned, and materials were assayed for drug carryover. Sulfamethazine was detected in the mixer clean-out material at concentrations of 12.6 ppm and 8.1 ppm for the granular and pellet forms, respectively. Carbadox carryover was not detected in mixer clean-out material. Detectable concentrations for all three drugs occurred in clean-out material from the leg and sack-off bin.*

Concern over the safety of the food supply in the United States is paramount among consumers. The current good manufacturing practices (cGMPs) used to regulate animal feed production outline procedures to help assure that meat, milk, and eggs produced from animals receiving medicated feeds contain no violative drug residues. Food and Drug Administration (FDA) cGMPs specify that "equipment shall be capable of producing medicated feed of intended purity and potency"[1]; this includes proper mixer performance. Mixer testing procedures are outlined by the American Society of Agricultural Engineers (ASAE).[2] This procedure entails describing feed uniformity by calculating the coefficient of variation (CV) using salt assays from 10 feed samples collected from the mixer. The cGMPs also specify that "adequate procedures shall be established and used for all equipment used in the production and distribution of medicated feeds to avoid unsafe contamination of medicated and nonmedicated feeds."[1]

Sulfamethazine and carbadox are two antibacterial drugs widely used in swine production. Residue tolerances for these two products in uncooked tissue are 0.1 ppm and 0.0 ppm, respectively.[3] Both products are classified as category-II drugs under the cGMPs; withdrawal times are 15 days for sulfamethazine and 10 weeks for carbadox. Both products are used to improve weight gain and feed efficiency, as well as to control or prevent bacterial diseases.[4]

The high rate of violations for tissue residues of sulfamethazine has concerned FDA personnel for years.[5] The FDA has identified that a lack of sequencing, flushing, and cleaning of mixer equipment accounted for 25% of sulfamethazine violations. As little as 1 ppm of sulfamethazine in feed, or 1/4 teaspoon of sulfa in a 908-kg batch of feed, can cause violative sulfa residues.[6] Evaluating how these two medicated feed additives perform when flushing and cleaning out feed manufacturing equipment may help explain how feed becomes cross-contaminated.

Studies examining the cause of cross-contamination in feed manufactured on-farm revealed that powdered sulfamethazine increased this risk compared to the granular form of the drug.[7,8] The drug manufacturing industry developed granular and pelleted forms of sulfamethazine to help reduce cross-contamination. This effort, combined with a strong education campaign by the USDA and the FDA, reduced the violation rate in pork from 13% prior to 1978 to about 5% between 1980 to 1987.[5] For sulfamethazine in swine, the current residue violation rate is less than 1%.[9]

Improper mixing and incorrect inclusion rates of medicated feed additives create the potential of tissue residue violations. The FDA has established acceptable assay error ranges of 20% and 25% for complete feed containing sulfamethazine and carbadox, respectively.[10] Exceeding these error ranges presents a potential source of violative tissue residue, whereas inclusion rates below the established error range may reduce the efficacy of the drug to control disease and allow microbial resistance to develop.[6]

This study was conducted to examine the mixing and clean-out properties of two forms of sulfamethazine and one carbadox product to better understand the role that product form, mixing performance, and flushing/clean-out properties may play in producing quality feed.

TJH, KCB: Department of Grain Science and Industry; TML: Department of Statistics, Kansas State University, Manhattan, Kansas, 66506-2201.

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Methods

Medicated swine feed was produced in 454-kg batches at the feed mill (Figure 1) of the Department of Grain Science and Industry, Kansas State University (Table 1). The study was replicated three times for each of three Category-II Type-B medicated feed additives[10]:

- 22 g per kg sulfamethazine in extruded pelleted form (i.e., "pelleted sulfa"),
- 22 g per kg sulfamethazine in granular form (i.e., "granular sulfa"), and
- 5.5 gper kg carbadox in granular form (i.e., "carbadox").

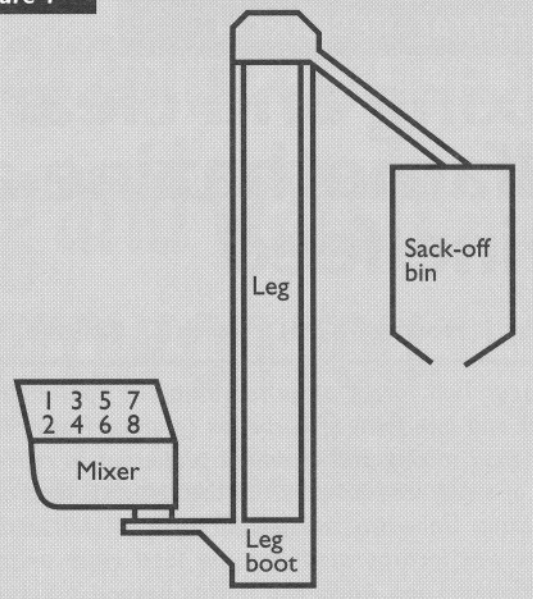
Sulfamethazine was included in the feed at a rate of 110 mg per kg (110 ppm) of feed, and carbadox was used at 55 mg per kg (55 ppm) of feed. Treatments were arranged in a completely randomized design with repeated measures taken at three mixing times and after transferring feed to 22.7-kg sacks.

Corn conforming to United States Grain Grading Standards for number 2 yellow corn was ground to a particle size ranging between 550 and 700 microns using a Jacobson hammermill with a 0.317 cm (1/8 inch)-diameter screen. A 182-kg ground corn placebo was passed through the mixing and sack-off system. Then, the mixer, leg, and sack-off bin were cleaned prior to mixing feed for the study. The mixer and leg boot were cleaned using separate shop vaccuums. A different vacuum filter was used for each drug treatment.The sack-off bin was cleaned and the sample collected from this area by striking it with a rubber mallet and collecting the material that was knocked off by the resulting vibration. Feed consisting of corn (73.5% by weight) and soybean meal (22.2% by weight) was batched with a Wisconsin Electric Manufacturing, Inc., system and emptied into a Sprout Waldron horizontal double-ribbon mixer. The micro-ingredients (monocalcium phosphate, limestone, lysine, vitamins, trace minerals, and salt) were added to the mixer by an Able micro-ingredient system. The medicated feed additives were applied by hand after the grain, protein, mineral, and micro-ingredients were added to the mixer.

Mixing properties of the medicated feed additives were evaluated by sampling the mixer using a Seedburo Grain Probe (Chicago, Illinois) after 1.5, 2.5, and 4 minutes of mixing time. In order to reduce costs to incorporate replications, the CVs were computed based on eight rather than 10 samples. Following the mixing treatment, feed was conveyed to the sack-off bin and packaged into 22.7-kg capacity sacks, of which eight were sampled. Two flush treatments with 91 kg of corn followed each batch of feed. The feed system was cleaned by the same procedures used in mill preparation.

Samples from the mixer, packaging, flush, and clean-out were split using a riffler and analyzed separately for salt and drug content. Salt analyses were performed using Quantab titrators (Elkart, Indiana). Drug assays for sulfamethazine and carbadox were performed by a commercial lab following Association of Analytical Chemists (AOAC) methods.[12] The lowest detection limits in feed samples for these two assays are 5 ppm and 2 ppm, respectively. Triplicate assays were performed on all samples that were 30% outside the desired medication concentration following the first assay results.

Figure 1



Sketch of feed mill. Samples taken from locations numbered 1–8.

Table 1

Swine grower ration used to test mixing and clean-out properties of sulfamethazine and carbadox.

Ingredient	Weight (kg per 1000 lb of feed)
Corn	333.49
Soybean meal 48%	100.79
Monocalcium phosphate	6.58
Limestone	4.81
Lysine 98%	0.45
KSU vitamin pack	0.91
KSU trace mineral	0.68
Salt	1.36
Medicated feed additive	
Pelleted and granular sulfa*	2.27
Carbadox	4.54

*An additional 2.27 kg ground corn was added to the feed containing the sulfa.

Statistical analysis

Coefficient of variation, standard deviation, and mean measurements taken across the locations were calculated for each drug, replication, and mixing time using the Univariate procedure in SAS.[12] Drug concentrations were analyzed on a proportional basis because the carbadox inclusion rate was half of the sulfamethazine inclusion rate. The GLM procedure in SAS was used to evaluate treatment effects for both the mixing and clean-out portions of the study. Main effects were separated using Fisher's

least-significant-difference technique, and interactions were analyzed using the least-significant-difference among the least squares means produced by the GLM procedure. Variance components in the general linear model were evaluated using the VARCOMP procedure of SAS. A paired-comparison T-test was performed on the mean difference between drug mixing uniformity and salt mixing uniformity.

Results and discussion

Mixing properties

Mixing properties compared among drugs were different ($P<0.01$), whereas mixing time compared among drugs did not differ ($P>0.05$). Carbadox mixed well, as indicated by an average CV of 11.4% (Table 2). THe CV for pelleted sulfa was 30.4%, while the CV for granular sulfa was 25.6%.

Increased mixing time after 1.5 minutes did not improve the uniformity of drug distribution in the swine feed ($P > 0.1$). This suggests that some factor other than mixing time hindered sulfamethazine distribution in the feed. Electrostatic properties of feed ingredients are reported to occur;[13,14] however, a paucity of information is available regarding the influence of static charge on mixing properties of feed ingredients. Ingredient carriers, oil, and grounding the mixer are used to reduce static cling. However, ingredients not directly in contact with the mixer may possess electrostatic charge. If static charge was the cause for non-uniform distribution of sulfamethazine in the feed, additional mixing would not rectify this problem. Further investigation to explain the cause for poor mixing performance should include measuring various physical properties of sulfamethazine, salt, and corn such as density, particle size, hygroscopicity, conductivity, and static charge during mixing or movement.

Mean assay values for each drug × mixing time combination (Table 2) indicate that the pellet form of sulfamethazine was present at a lower concentration (87.1 ppm) than the granular form (108.4 ppm) in the complete feed. Both sulfamethazine products were packaged as a Type-B premix at a concentration of 22 g per kg (10 g per lb), and assays of the premixes for drug concentration indicated that the granular and pellet forms contained 114% and 104% of the label, respectively. The higher mean for the granular sulfamethazine explains why its CV was smaller than that of the pellet form. The standard deviations for both products were similar, and the range between assays was about 27 ppm greater for the granular product.

Table 2

Coefficient of variation (CV) percentage and mean, range, and standard deviation (in ppm) for two sulfamethazine forms and carbadox at 1.5, 2.5, and 4 minutes mixing time and after bagging feed.

Treatment	Assay results			
Pelleted sulfamethazine	CV %	Mean	Range	Std. Dev.
1.5 min. mix	28.2	73.6	86.9	20.8
2.5 min. mix	32.4	90.2	126.9	29.2
4.0 min. mix	30.8	98.6	122.6	30.4
bags	30.4	85.9	103.1	26.2
Average	30.4	87.1	109.9	26.6
Granular sulfamethazine				
1.5 min. mix	25.1	109.8	125.0	27.6
2.5 min. mix	25.9	112.7	150.5	29.2
4.0 min. mix	28.2	104.5	149.5	29.5
bags	23.4	106.6	121.8	25.0
Average	25.6	108.4	136.7	27.8
Carbadox				
1.5 min. mix	14.3	48.1	30.5	6.9
2.5 min. mix	5.7	44.5	10.7	2.6
4.0 min. mix	10.6	45.2	18.6	4.8
bags	14.9	44.7	31.0	6.7
Average	11.4	45.6	22.7	5.2

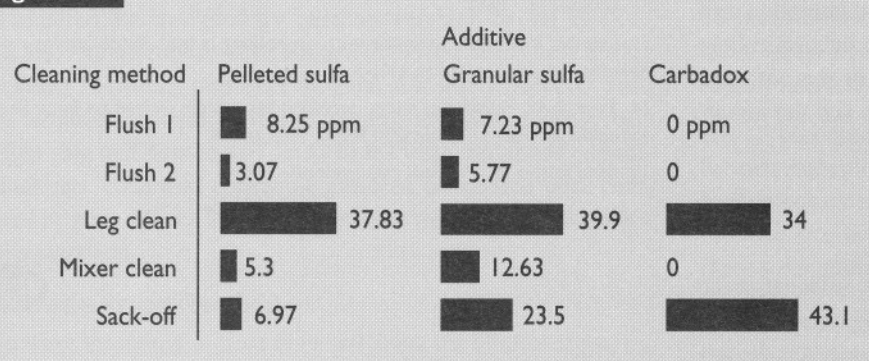
The statistical components of variability for the two sulfamethazine products and carbadox were analyzed using data from samples subjected to triplicate drug assays. Assay variability was small relative to other components of variability in the experiment. The greatest variability occurred between replications for the same sample site within each drug treatment.

The paired-comparison T-test between salt and drug CVs revealed that carbadox did not differ ($P>0.05$) from salt with respect to distribution uniformity in the feed, whereas both forms of sulfamethazine displayed mixing properties that differed ($P<0.05$) from those of salt. Hasselberger[15] reported a similar observation with regard to mixing properties of chlortetracycline, penicillin, and sulfamethazine in feed concentrates.

Clean-out properties

We observed a drug × location interaction in feed clean-out/flush material ($P<0.01$) (Figure 2). Drug concentrations in both the ground-corn flush treatments did not vary

Figure 2



Interaction between three medicated feed additives and five sources of clean-out material.

($P>0.05$) among products. A trend, in which sulfamethazine was present in flush material whereas carbadox was not present at detectable concentrations, was established for both corn flush treatments.

The mixer clean-out samples displayed a similar trend as the ground corn flush with respect to drug carryover (Figure 2). Sulfamethazine content in mixer clean-out samples did not differ ($P>0.05$) between the pelleted (8.1 ppm) and granular (12.6 ppm) product, whereas carbadox (<2.0 ppm) differed from the granular form of sulfamethazine ($P<0.05$). The highest sulfamethazine concentration (16.2 ppm) found in 1.1 kg of mixer clean-out material could result in a contamination of 32 parts per billion in the subsequent 454-kg batch of feed. This is below the 1 ppm concentration that can lead to violative tissue residues.[5]

The feed collected from the boot of the leg contained higher drug concentrations than the flush and mixer clean-out material ($P<0.05$). No difference was observed among the three drug products. The highest concentration of sulfamethazine carryover (37.8 ppm pellet and 39.9 ppm granular) occurred in the material collected from the boot of the leg. Since this is a dead spot in the feed conveying system, the only way to remove carry-over material is to clean the boot (physical removal). A high concentration of drug at this location is not undesirable, since the pellet and granular products were designed to flush from the system.

The concentration of drug in material collected from the sack-off bin varied dramatically between products. The concentration of carbadox in the sack-off bin was approximately 86% of the inclusion rate (43 ppm) compared to the sulfamethazine products, which were present at 7 ppm (6.4%) for the pelleted form and 23.5 ppm (21.4%) for the granular form. Clean-out material from the sack-off bin consists of fine, dust-like particles. Perhaps carbadox possesses similar dust-like properties and separates from the feed at the sack-off bin. The presence of a high drug concentration in the sack-off bin appears particularly hazardous as it is likely to result in product cross-contamination. The high carbadox concentration in the sack-off bin may also explain why it was not present in the ground corn flush.

Veterinarians should be aware of the different properties that medicated feed additives possess with respect to mixing and clean-out performance. In light of these results, it is imperative that the cGMPs are followed to avoid cross-contamination and violative tissue residue. Veterinarians can play an integral role in cautioning producers who mix their own feed of the potential hazards associated with medicated feed additives and the importance of good manufacturing practices.

Implications

- The two medicated feed additives containing sulfamethazine did not incorporate uniformly in the feed. The cause of the poor mixing performance of sulfamethazine was not discovered; however, assay variability was eliminated as a primary source of variation.
- Salt is not necessarily a good model for mixing and clean-out properties of in-feed medications. A paired-comparison T-test of the CVs from salt assays and CVs of the drug assays showed that sulfamethazine did not perform in a comparable manner to salt during the mixing process.
- Flushing the feed mixing, conveying, and sack-off systems twice with ground corn did not eliminate drug carryover. The granular form of sulfamethazine was present in mixer clean-out material at an average concentration of 13 ppm. Carbadox was present in the clean-out material from the sack-off bin at a concentration nearly equal to the inclusion rate.
- Study results indicate that further investigation of the mixing and clean-out properties of medicated feed additives is warranted.

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