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Evaluation of *Salmonella* Shedding in Cattle Fed Recycled Poultry Bedding

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Abstract

Recycled poultry bedding (RPB), contaminated with salmonella, was fed to beef calves to determine if it would increase the prevalence of detectable salmonella fecal shedding. Sixty Angus crossbred steer calves were placed on balanced rations containing salmonella contaminated recycled poultry bedding that had been properly or improperly stacked, or fed a control diet for an 84-day growing phase. After the growing phase, the calves were transported 12 hours to simulate shipping stress and then fed a single finishing diet. Fecal samples were collected from each calf and cultured for salmonella prior to the start of the trial, every 14 days during the growing phase, 24 hours after transport and every 28 days during the finishing phase. At the end of the finishing phase, scrapings from the ileocecal mucosa were collected at the abattoir and cultured. Dietary components and total mixed rations were sampled and cultured weekly for salmonella. Other than the poultry bedding at delivery, none of the dietary components or calves were culture-positive for salmonella at any time during the feeding periods or after transport. One calf that had been on a RPB diet during the growing phase was positive for *Salmonella norwich* at postmortem collection; however, it was not established that this was the same serotype of salmonella cultured from the RPB. We conclude that feeding a known salmonella contaminated feed source as a part of a balanced ration did not increase the prevalence of detectable salmonella shedding in calves over the published prevalence.

Résumé

De la litière recyclée de poulailler contaminé avec *Salmonella* a été ajoutée à la ration de veaux d'engraissement pour voir si l'ajout allait amener une hausse de la prévalence d'excrétion de *Salmonella* dans les fèces des veaux. Un total de 60 bouvillons Angus de race hybride ont été nourris soit avec un régime équilibré contenant de la litière recyclée de poulailler contaminé avec *Salmonella* et qui avait été entreposée de façon adéquate ou non, ou soit avec un régime témoin pendant un période de 84 jours durant la période de croissance. Après la période de croissance, les veaux ont été transportés pendant 12 heures pour simuler le stress du transport et nourris finalement avec un seul régime de finition. Des échantillons fécaux ont été recueillis de chaque veau et mis en culture pour vérifier la présence de *Salmonella* à tous les 14 jours pendant la période de croissance. Des échantillons étaient aussi recueillis 24 heures après le transport et à tous les 28 jours pendant la phase de finition. A la fin de la phase de finition, des écouvillons de la muqueuse iléo-cæcale étaient recueillis à l'abattoir pour l'analyse

bactériologique. Les composantes alimentaires et la ration totale mélangée étaient échantillonnées pour vérifier la présence de *Salmonella* à chaque semaine. Mis à part les éléments de litière au départ, aucune des composantes alimentaires ni aucun des veaux n'ont été positifs aux salmonelles durant les périodes d'alimentation et après le transport. Un veau qui avait reçu le régime avec litière recyclée durant la phase de croissance a été positif pour *Salmonella norwich* au moment de l'examen post-mortem. Toutefois, on n'a pas pu déterminer si le sérotype de cette bactérie était le même que celui cultivé à partir de la litière. Nous concluons que l'ajout de matériel contaminé avec *Salmonella* à un régime équilibré n'augmente pas la prévalence d'excrétion de *Salmonella* dans les fèces des veaux au-delà de celle établie dans la littérature.

Introduction

Bacterial foodborne diseases cost billions of dollars annually because of treatment costs, large productivity losses and untimely death of people affected.[49,50] Because of the losses associated with foodborne illness, several governmental agencies are attempting to identify major risk factors and ways to decrease food product contamination by animal origin pathogens.[3] One report concludes that a primary contamination source at the farm level is poor feed and farming practices, particularly mismanagement of animal manures.[2] salmonella contamination of feed products (particularly by-product feeds) is not uncommon.[4,15,27,43,51]

Recycled poultry bedding (RPB), or poultry litter, is an important feed resource to the cattle industry in the southeastern United States to supply protein, energy and minerals. Because salmonella is prevalent in poultry production,[15] there has been concern in the popular press regarding how this practice may be contributing to the amount of salmonella and other potential pathogens in cattle, which are consequently more likely to spread the organism to humans *via* meat contamination at harvest.[45] In the poultry industry, bedding has been examined as an indicator of flock salmonella infection. In these studies, old bedding (as is used in cattle feeding) has been noted to be 'salmo-nellacidal.'[6,16,42,47,48] Several studies have shown that heating RPB in some manner reduces salmonella growth.[1,10,26,28,37,39,40] Based on this research, it would appear that the likelihood of salmonella spread through feeding RPB would be minimal, although no studies have been conducted to determine the actual risk. This study was designed to investigate whether feeding cattle RPB, which was naturally contaminated with salmonella upon delivery from the poultry producer, would increase the prevalence of the organism in feces.

Materials and Methods

Animals

Sixty Angus-cross calves were purchased from North Carolina graded feeder calf sales.[41] Calves arrived in the evening and were processed the next morning. All calves received vaccination against *Mannheimia (Pasteurella) haemolytica* type A, clostridial diseases and respiratory viruses using a modified live virus (MLV) vaccine. Calves were dewormed with injectable ivermectin (label dosage) and eartagged for individual identification. Calves were administered a second viral respiratory vaccine (MLV) 4-6 weeks later.

Calves were maintained on a mixed pasture (bermuda, fescue and crabgrass), with bermuda and fescue hay supplied free choice for two months prior to the start of the feeding trial to allow acclimation, ensure health and await availability of individual animal feeding facilities. A commercial trace mineralized salt block was available free choice. Calves were dewormed with injectable ivermectin again upon removal from pasture to eliminate intestinal parasites before the feeding trial began.

Experimental Design

The calves were blocked by weight (range of 544 to 705 lb; 247-320 kg) and sorted into five pens of 12 animals each. Within each pen, two animals were randomly assigned by drawing numbers to each of the six treatments. This totaled 10 animals for each of the six treatments. Calves were allowed two weeks to acclimate to the pens and individual feeders[a] before starting the feeding trial. The pens were covered and had slatted, concrete flooring. Each pen had one or two community waterers and 12 individual feeding gates accessed *via* a magnetic transponder suspended from each calf's neck. Before the calves were moved into the pens, the area was power–washed to remove as much environmental contamination as possible. Each pen, waterer and feeding gate was swabbed and cultured for *Salmonella* spp.

Treatment Diets

The experiment was a 2x3 factorial design with the factors being diet type and the presence (+M) or absence of monensin (30 g/ton of diet DM). There were three diet types: control (CON), deep-stacked RPB (DS-RPB) and shallow-stacked RPB (SS-RPB). CON diets contained 33% corn silage (SIL), 33% cottonseed hulls (CSH), 22% corn and 11% soybean meal (SBM) on a dry matter (DM) basis. A mineral supplement, balanced for growing cattle, was also added to CON diets. The RPB diets contained 15% SIL, 35% corn, 15% CSH and 35% of the appropriate type of RPB on a DM basis. No additional mineral supplements were added to RPB treat-

ments. Diets were formulated to achieve a weight gain of 2.0 lb (0.91 kg)/day in steers of this age and type.

Recycled Poultry Bedding

A poultry producer was located who needed old bedding material removed from broiler houses. Three flocks of broilers had been raised on the bedding prior to removal. Prior to removing the bedding, the three houses were sampled for salmonella contamination using the drag swab technique. To perform this technique, a modification of the procedure described by Mallinson *et al.*[35] was used. The swab was dropped onto the bedding surface and the string was carefully unrolled to avoid contamination from the operator's hands. The swab was then dragged back and forth across 1/3 of the surface of the bedding for 10–15 minutes. Three swabs were used on each house to sample the entire building. The swab was then placed into a sterile container (string removed) and returned to the laboratory for culture.

Immediately following removal from the poultry house, the *Salmonella*-positive RPB was delivered to the research facility. Upon delivery, representative grab samples from each house were collected. Three replicates of 10-gram RPB subsamples were cultured to ensure salmonella was still present. Two stacks of bedding were made, with bedding from all houses included in each stack. Both stacks were placed on concrete in a commodity shed (walls on three sides and a roof). The deep stack was approximately 7.5 ft (2.3 m) high at its peak. During stacking, the DS-RPB was compressed with a loader to limit oxygen. This stack was covered with 6 mil plastic and allowed to heat, as per industry recommendations.[44] The shallow stack, was heaped to approximately 3 ft (0.9 m). No attempts were made to compress the SS-RPB and it was not covered. During stacking, temperature probes were inserted throughout the central areas of the stacks to monitor heating. The stacks were undisturbed for 51 days before the feeding trial began (minimum recommended time is 21 days).[44] The extended length of time the stacks were undisturbed was based on the difference between RPB delivery date and individual animal feeding facility availability.

Sampling of Diets

Grab samples of the total mixed ration (TMR) for each treatment, as well as DS-RPB, SS-RPB, SIL, CSH, concentrate mixes and mineral components were collected weekly for nutrient analysis. Representative 25-gm subsamples of these were transferred to a sterile resealable container and cultured for salmonella. Clean latex gloves were worn during sample collection and were changed between samples to avoid cross-contamination.

Study Period

Growing Phase - Fecal samples were collected from all calves within seven days of arrival at the research facility. Calves were resampled two weeks prior to trial initiation (three days after having been moved to the feeding pens). Resampling at this point was an attempt to identify any calves that were salmonella carriers, as a stressful situation such as moving and establishing a new social hierarchy can induce fecal shedding of the organism.[31]

Calves were fed the treatment diets for 84 days. Calf nutritional performance parameters (weight gain, feed intake, gain:feed ratio) were recorded throughout the trial period. Starting on the first day of the trial (day 0), fecal samples were collected from each calf every 14 days. The 14-day interval was selected based on economic and time constraints. Fecal samples were obtained manually *per rectum*. Each sample was collected using a fresh latex glove to avoid contamination between calves. Samples weighed from 2.5 gm to over 100 gm, depending upon whether the animal had recently voided its rectal content. Each sample was collected into a sterile container and placed in a dark, temperature-controlled container for transport to the laboratory. At the laboratory, 10 gm of feces (or the entire sample, if less than 10 gm was available) were transferred to a sterile container for culture, as described below.

Transport Stress - At the end of the growing phase, the calves were transported on a standard livestock truck for six hours, unloaded and maintained in an unfamiliar drylot overnight, then returned to the original facility. The calves were in one large, intermixed group in the drylot, rather than in the smaller groups assigned during the growing phase. This protocol has been previously shown to induce stress at this research site.[30] The stress induced by this protocol was intended to identify any cattle that were chronic carriers, but not detected as shedding salmonella during the growing phase. Stress-induced shedding of salmonella has been documented in cattle that are chronic carriers of the organism.[13] All calves had access to water and a small amount of grass hay while held overnight in the drylot. This transport period was used to simulate movement from a grower operation to a finishing operation. Twenty-four hours after return to the research farm, fecal samples were collected for culture from all calves, as previously described.

Transition Period, Finishing Phase and Harvest - After transport, the animals were replaced in their original pens, transitioned to a finishing diet for 14 days and fed identical finishing diets for 120 days. The finishing diet contained, on an as-fed basis, 10% CSH and 90% concentrate. The concentrate contained 92.35% corn, 5.45% SBM, 1.1% limestone, 0.55% trace mineral salt, 0.55% urea, 187 gm Rumensin 80® (Elanco Animal Health), 187 gm of a vitamin A, D and E mixture (2200 IU/kg A, 275 IU/kg D, and 30 IU/kg E) and a trace min-

eral mix. Fecal sampling and culturing was performed every 28 days during the finishing phase, starting on the first day of the finishing phase. Again, the 28-day interval was based on economic and time constraints.

When cattle were visually judged to be adequately finished, they were transported to a commercial abattoir in two shipments, with equal numbers of calves per treatment group shipped each time. Calves were held overnight at the abattoir prior to processing. Calf identification was maintained through harvest and the ileocecal junction of the gastrointestinal tract was collected. The intestinal samples were transported back to the laboratory. This section of intestinal tract was opened lengthwise and the mucosa was scraped to remove the content for culture.

Microbiological Protocols

Once placed in the sterile, resealable container, all samples collected for culture were processed in the same manner. Ninety grams of 1% buffered peptone water (BPW) were added and the sample was manually agitated. The solution was placed in an incubator at 99°F (37°C) for 20–24 hours. One-hundred microliters of the fluid portion from each sample was then transferred to a test tube containing 9.9 ml of Rappaport-Vassiliadis (R10) broth for salmonella enrichment and incubated at 99°F (37°C) for 24 hours. Ten microliters of the R10 broth solution was then streaked onto xylose-lysine-tergitol (XLT4) agar plates,[36] inverted and incubated at 99°F (37°C) for 24 hours. Presence or absence of colonies characteristic of salmonella was noted (convex, black centered colony with a clear, smooth edge). Any suspect colonies were confirmed biochemically as described below.

At each sampling time, sterile BPW served as a negative control. Positive controls consisted of a loop full of salmonella from a laboratory isolate added to sterile BPW, and a randomly selected calf fecal sample in BPW seeded with the same salmonella isolate.

Suspect colonies were placed on urea and Triple Sugar Iron (TSI) slants to confirm biochemical reactions indicative of salmonella. A positive reaction on a urea slant is indicated by a color change to bright pink. Salmonellae are urease negative and therefore no color change would be expected on this medium. TSI incorporates several factors for identification of bacteria. The medium contains glucose, lactose and sucrose. Positive TSI reactions for salmonella are alkaline on the slant surface, acid in the butt of the tube, production of H_2S and variable gas production.[34]

To rapidly identify a *Salmonella*-positive source of RPB for purchase, initial RPB samples were biochemically analyzed using the VITEK Jr.®.[b] The VITEK Jr.® performs 30 biochemical reactions to yield a profile that is then matched to an appropriate organism. Positive samples were stored at 39°F (4°C) for later serotyping.

Fecal samples that yielded positive results on urea and TSI were shipped overnight on ice to a USDA facility for serogrouping based on monoclonal antibody reactivity.[c] After serogrouping, salmonella organisms were shipped overnight on ice for serotyping by the National Veterinary Services Laboratory.[d]

Sensitivity of Microbiological Protocol

The method used for culturing salmonella was tested for sensitivity in identification of salmonella organisms. Serial dilutions of a known amount of a laboratory strain of *Salmonella typhimurium* were added to ten replicates of calf fecal samples and subjected to the protocol described above.

Results

Sensitivity of Microbiological Protocol

Serial dilutions of a known amount of *Salmonella typhimurium* demonstrated that when 40 CFU/gm were inoculated into a random fecal sample, salmonella could be isolated from 80–85% of samples at the end of the protocol period.

Salmonella in Recycled Poultry Bedding

Little historical information on the bedding was available. On visual inspection, the RPB appeared to be wood shaving based. Other than drag swabbing to identify salmonella contamination, no other measurements (e.g. depth) were taken on bedding while it was in the house.

Samples (five of nine) from the bedding collected by drag swabbing and one sample (of three) collected upon delivery to the research facility were *Salmonella*-positive based on biochemical analysis using the VITEK Jr.® system. Samples sent for serogrouping were contaminated with other bacteria, so grouping was not possible. Attempts to re-isolate the organism from the original sample were unsuccessful.

Temperature measurements from the center of the stacked bedding showed that the deep stacked bedding reached 122°F (50°C) by 20 days into the stacking period and stayed above that temperature until feeding started. One area of the shallow stacked bedding reached 104°F (40°C) on one day and then dropped to below 95°F (35°C) for most of the remainder of the study.

Dietary Components

Salmonella was not isolated from the RPB post-stacking or from any of the weekly grab samples of TMR's, DS–RPB, SS–RPB, CSH, SIL, concentrate mixes or mineral components during the trial.

Pens

No salmonella was isolated from the pens, waterers or feeding gates after powerwashing and prior to placement of calves into the pens.

Calves

Fecal samples collected from calves during the growing phase, 24 hours after transport, and during the finishing phase were all negative for salmonella. One calf from each treatment group was not transported due to weight restrictions on the livestock truck; however, these calves were sampled at the 24-hour post-transport time-point with the other calves. Salmonella was isolated from scrapings of the ileocecal mucosa taken from one calf (DS-RPB diet) at harvest. This isolate was serotyped *Salmonella norwich*. This results in a prevalence of 2.5% (1 of 40 animals on a RPB diet).

Calves in all treatment groups gained weight and converted feed at an acceptable level, although there were differences noted in their performance.[9]

Discussion

Salmonella in Recycled Poultry Bedding

Swabs from two of the three houses were cultured positive for salmonella. Salmonella was identified in the RPB by VITEK Jr.®, but the sample became contaminated with multiple other bacteria and salmonella serotyping was not possible.

The temperatures reached in the center of the stack of DS-RPB are consistent with the thermal death threshold of Salmonella, while the SS-RPB temperatures were below this threshold.[15] Also, related studies by the authors resulted in a marked reduction in the number of viable salmonella in RPB, whether deep or shallow stacked for only 21 days, making it unlikely that salmonella would survive to be consumed by the cattle.[8]

Salmonella in Calves

The *Salmonella*-positive calf was fed DS-RPB during the growing phase. Feces from this calf were not positive for salmonella at any time during the antemortem collection period; only the postmortem ileocecal mucosal scraping was *Salmonella*-positive. All other cultures of calf feces and ileocecal mucosal scrapings were negative for salmonella.

It is not possible to definitively determine the origin of the salmonella isolated from the calf in this study since the serotype of the RPB isolate was unavailable. A comparison of the thirty biochemical reactions performed by the VITEK Jr.® shows one difference between the isolate from the RPB and the isolate from the calf. Although this is an apparently small difference, salmonella are very similar (most serovars being of one species), so just one difference in the biochemical reactions strongly suggests the presence of two different salmonella, with the calf isolate likely from a different source than the RPB isolate. *Salmonella norwich* is rarely isolated; however it has been seen in both poultry and cattle,[17-24] so the RPB cannot be ruled out as the source of contamination in this calf. To definitively rule out the RPB as the source of the salmonella in the calf, both isolates would have to be serotyped or analyzed genetically.

It is possible that the *Salmonella*-positive animal was a carrier. It has been well established that cattle can be subclinical carriers of pathogens, such as salmonella.[5,29,46] Stressful events can lead to the development of disease by organisms present in the animal or by increased susceptibility to outside pathogens. A stressful event includes any change in the environment of the animal. Feed withdrawal, diet change, weather change, processing, transporting and mixing animals are all examples of stressful events.[13] Normal marketing channels for cattle include most of these events. The stress associated with regrouping the calves into pens prior to the start of the growing phase could be expected to cause fecal shedding of salmonella, if it was present. However, no salmonella was found at this point. Several studies have assessed the stress associated with transporting cattle.[12,14,31] The stress associated with transport can induce salmonella carriers to actively shed the organism in their feces.[13] Calf transport at the end of the growing phase was performed specifically to induce stress-related fecal shedding of salmonella, if present. The calf that was *Salmonella*-positive at slaughter was transported after the growing period and was not shedding detectable numbers of salmonella after transport. Either these episodes were not stressful enough to induce shedding, or the animal was shedding low, undetectable numbers of organisms. One possibility is that the calf became infected after the end of the growing phase, perhaps from exposure to an infected rodent or bird or at transport, but did not shed organisms.

There is also a slight possibility that salmonella was not recovered in an animal that was actually positive because of the length of the sampling intervals. Ideally, fecal samples would have been collected daily throughout the entire trial. Economic and time constraints made this level of sampling impractical. To account for this deficiency, calves were cultured at the shortest feasible intervals and specifically cultured after periods of stress to increase the likelihood of obtaining positive cultures.

It is important to note that the presence of salmonella in the gut does not necessarily mean it was present in the feces of the calf. Fecal samples taken during the feeding period did not show salmonella contamination. It would have been desirable to test fecal samples or hide swabs collected at the abattoir, just prior to harvest, but logistical difficulties prohibited fecal sample collection from the calves while in the holding pens. As salmonella carcass contamination generally occurs from fecal contamination of the hide, its presence in the gastrointestinal tract, rather than the feces, is of little consequence from a food safety perspective.[25] Further

studies assessing fecal shedding of salmonella while in the holding pens at the abattoir are warranted to assess this risk.

Related studies by the authors resulted in a marked reduction in the number of viable salmonella in RPB, whether deep or shallow stacked for only 21 days, making it unlikely that salmonella would survive to be consumed by the cattle.[8] Although this is compelling evidence that a feeding trial may not be necessary, this project was conducted to ascertain if there might be other, undetermined factors that influenced salmonella shedding in the calves fed RPB diets.

Furthermore, studies on the ability of salmonella organisms to survive in ruminal fluid indicate that survival is limited at the pH and volatile fatty acid concentrations typically seen in the rumen of healthy cattle on feed.[7,11,38] The ruminal fluid pH and volatile fatty acid concentrations were measured in the calves in the present study at the end of the growing phase (data not shown) and were consistent with the inhibitory values reported in those studies. Thus, it is very unlikely that feeding RPB would increase the prevalence of salmonella shedding in animals fed RPB-based diets, provided appropriate handling recommendations are followed. Overall, there was no higher prevalence of salmonella infection in the RPB-fed animals than that expected for animals fed other diets in other environments.

Conclusions

Only one of 40 calves fed RPB was positive for salmonella, which was a prevalence of 2.5%. Prevalence estimates of salmonella infection in cattle vary. Two studies of dairy calves reported that just over 2% were shedding salmonella, regardless of their health status.[32,33] Figures released in 1995 by the USDA indicated that 5.5% of fecal samples from feedlot cattle were *Salmonella*-positive.[49] The calves in this trial had a prevalence within the range generally recognized in the literature, and no detectable shedding of salmonella ante-mortem. We conclude feeding a diet containing *Salmonella*-positive RPB that has been stored for a period did not result in increased prevalence of salmonella shedding among calves regardless of heating of the RPB. Therefore, the risk of carcass contamination with salmonella and potential for a salmonella foodborne disease outbreak from calves fed RPB is considered to be minimal. Although RPB was stacked for 51 days prior to feeding in this study, the authors continue to recommend only a 21-day minimum period of deep stacking prior to feeding RPB. Data collected by the authors (not presented) establishes this period as adequate to eliminate the majority of salmonella organisms present. This study did not evaluate the effect of feeding RPB directly out of the poultry house, and the authors discourage this practice.

Footnotes

[a]Calan Gate Feeders, American Calan, Inc, Northwood, New Hampshire.
[b]bioMerieux Vitek, Inc., Hazelwood, Missouri – Gram-Negative Identification Card for *In-Vitro* Diagnostic Use Pinsert (Rev 0498 / AU).
[c]Laboratory of Dr. James Keen, USDA–ARS, U.S. Meat Animal Research Center, Clay Center, Nebraska.
[d]National Veterinary Services Laboratory, USDA-Animal and Plant Health Inspection Service, Ames, Iowa.

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