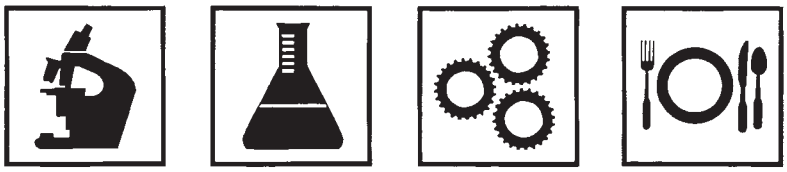
Journal of Food Protection



Survival of *Enterococcus faecalis* OG1RF:pCF10 in Poultry and Cattle Feed: Vector Competence of the Red Flour Beetle, *Tribolium castaneum* (Herbst)†

LAKSHMIKANTHA H. CHANNAIAH,[1] BHADRIRAJU SUBRAMANYAM,[1]* AND LUDEK ZUREK[2,3]

[1]*Department of Grain Science and Industry,* [2]*Department of Entomology, and* [3]*Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, Kansas 66506-2201, USA*

Journal of Food Protection, Vol. 73, No. 3, 2010, Pages 568–573

Research Note

Survival of *Enterococcus faecalis* OG1RF:pCF10 in Poultry and Cattle Feed: Vector Competence of the Red Flour Beetle, *Tribolium castaneum* (Herbst)†

LAKSHMIKANTHA H. CHANNAIAH,[1] BHADRIRAJU SUBRAMANYAM,[1]* AND LUDEK ZUREK[2,3]

[1]*Department of Grain Science and Industry,* [2]*Department of Entomology, and* [3]*Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, Kansas 66506-2201, USA*

MS 09-334: Received 3 August 2009/Accepted 11 December 2009

ABSTRACT

Laboratory experiments were designed to determine the survival of *Enterococcus faecalis* OG1RF:pCF10 in poultry and cattle feed and its acquisition and transmission by adults of the red flour beetle, *Tribolium castaneum* (Herbst), to sterile feed. Adult *T. castaneum* beetles were introduced into poultry and cattle feed inoculated with *E. faecalis* OG1RF:pCF10 and incubated at 28°C with 65% relative humidity for 7 days in a growth chamber. *E. faecalis* survived in both poultry and cattle feed during the 7-day test period. There was a logarithmic decrease in *E. faecalis* concentration in poultry and cattle feed and in and on the insects. *E. faecalis* persisted on the surface and within *T. castaneum* adults for 7 days when adults were released on *E. faecalis*–inoculated poultry feed and for only 5 days on *E. faecalis*–inoculated cattle feed. The concentration of *E. faecalis* decreased more slowly on poultry feed than on cattle feed, and this may explain why adult *T. castaneum* insects were more successful in acquiring and transferring *E. faecalis* from inoculated poultry feed to sterile poultry feed during the 7-day test period. However, *T. castaneum* adults reared on inoculated cattle feed were unable to contaminate sterile cattle feed on day 7. To our knowledge, this is the first report documenting *T. castaneum* to successfully acquire antibiotic-resistant enterococci from animal feed and transfer them to sterile feed. Management of *T. castaneum* through effective integrated pest management program is therefore important to prevent the spread of antibiotic-resistant and virulent enterococci in animal feed and feed manufacturing environments.

Animal feeds are often contaminated with bacteria such as *Salmonella (15, 22), Enterococcus* spp. *(4, 30), Campylobacter* spp. *(25), Listeria* spp. *(21)*, and *Escherichia coli,* including *E. coli* O157:H7 *(20, 28)*. In addition to microbial contamination, animal feed can be infested by several stored-product insects *(17, 18, 24)*. These stored-product insects have been reported to harbor many potentially pathogenic bacteria. For example, the granary weevil, *Sitophilus granarius* (L.), a common pest of stored grains, is capable of transferring *Salmonella enterica* serotype Montevideo from contaminated wheat to fresh wheat *(14)*. In a similar study, *S. granarius* adults collected from laboratory colonies and grain storage facilities were identified as potential reservoirs for *Escherichia intermedia, Proteus rettgeri, P. vulgaris, Bacillus subtilis, Serratia marcescens, Streptococcus* spp., *Micrococcus* spp., and members of the *Klebsiella-Aerobacter* group *(11)*. The darkling beetle, *Alphitobius diaperinus* (Panzer), a stored-product pest generally associated with poultry brooder houses, was reported to harbor *Salmonella, E. coli, Campylobacter* spp. *(1, 13), Micrococcus* spp., *Streptococcus* spp., and *B. subtilis (5)*. The same insect species sampled from a turkey brooder house also carried *Streptococcus* spp. and *B. subtilis (12)*. The mealworm *Tenebrio molitor* (L.), a pest of poultry sheds and egg barns, is capable of transmitting poultry diseases *(16)*. Recently, antibiotic-resistant *Enterococcus faecium* was isolated from the red flour beetle, *Tribolium castaneum* (Herbst); the confused flour beetle, *Tribolium confusum* (Jacquelin du Val); the warehouse beetle, *Trogoderma variabile* (Ballion); and the maize weevil, *Sitophilus zeamais* (Motschulsky), associated with six feed mills located in the midwestern United States *(18)*. This is not surprising, considering the fact that enterococci have been previously isolated from feed samples *(4, 30)*.

Enterococci, which are ubiquitous in nature *(9),* have gained prominence in the last decade as an important reservoir of antibiotic resistance genes. Furthermore, enterococci can transfer their resistance traits to bacteria of greater clinical significance through horizontal gene transfer *(6)*. While enterococci with antibiotic resistance are associated with stored-product insects *(18),* it is not clear how these insects are contaminated with enterococci. We hypothesized that stored-product insects may be capable of acquiring enterococci from the feed and contribute to feed contamination in the feed manufacturing environments. In the present investigation, laboratory experiments were designed to evaluate the survival of *Enterococcus faecalis* in

* Author for correspondence. Tel: +1-785-532-4092; Fax: +1-785-532-7010; E-mail: sbhadrir@k-state.edu.

† Contribution 09-362-J of the Kansas Agricultural Experiment Station.

poultry and cattle feed and to elucidate the role of adult *T. castaneum*, a pest commonly associated with feed mills *(17)*, in the acquisition, retention, and transmission of enterococci from inoculated poultry and cattle feed to sterile feed.

MATERIALS AND METHODS

Poultry and cattle feed substrates. Poultry (12% moisture, wet basis) and cattle feed (11% moisture), freshly prepared in the Department of Grain Science and Industry's pilot feed mill, were used in this study. These feed materials are similar to poultry and cattle feed produced commercially. The ingredients in poultry feed include ground corn (70.0% by weight), soybean meal (20.4%), fishmeal (5.5%), soybean oil (3.0%), monocalcium phosphate (0.5%), limestone (0.6%), salt (0.3%), D-methionine (0.3%), L-lysine (0.1%), and poultry vitamin mix (0.25%). The poultry feed was batched by a Wisconsin Electrical Manufacturing automated batching system (WEM Automation Inc., New Berlin, WI). Batched feed was then mixed in a 500-kg Forberg double-shaft paddle mixer (Forberg International AS, Larvik, Norway), after which it was steam conditioned in a single-pass steam conditioner to a temperature of ~82°C, followed by pelleting in a California Pellet Mill (Master Model 1000, CPM Co., Crawfordsville, IN). A pellet die with 0.4-cm pellet diameter and 3.2-cm pellet thickness was used to make the pelleted poultry feed. The pelleted feed was cooled on a double-pass, perforated-bed, horizontal cooler before the pellets were bagged into 22.7-kg (50-lb) paper bags.

The ingredients in cattle feed include ground corn (77.0% by weight), cottonseed meal (5.2%), liquid molasses (4.0%), soybean oil (3.0%), ruminant vitamin mix (2.6%), dehydrated alfalfa (5.5%), and cotton seed hulls (3.0%). Cattle feed was batched, mixed, and bagged following procedures described for poultry feed, but the feed did not go through the pelleting process. The poultry feed and cattle feed had pH values of 6.5 and 7.5, respectively.

***E. faecalis* cultures and feed inoculation.** An overnight culture of *E. faecalis* OG1RF:pCF10 strain, resistant to tetracycline, grown in brain heart infusion broth (Difco, Becton Dickinson, Sparks, MD) at 37°C in an incubator with a shaker (Excel E24 incubator shaker series, Edison, NJ), was selected for this study. The bacterial cells were centrifuged, and the supernatant was discarded. The pellets were resuspended in phosphate-buffered saline (PBS; pH 7.2; MP Biomedicals, Solon, OH), diluted, and used to inoculate poultry and cattle feed. Poultry and cattle feeds, prior to inoculation, were ground in a mixer (KSM2 grinder, Braun, Kronberg, Germany) and then sifted through a sieve with opening dimension of 180 μm (Seedburo Equipment Company, Chicago, IL). The sifted material was dry sterilized at 121°C for 20 min. Poultry or cattle feed (100 g) was placed in 0.94-liter sterilized glass jars. The sample was inoculated with 5 ml of *E. faecalis* cell suspension. After inoculation, the feed material in each jar was shaken manually for 15 min to ensure thorough mixing of *E. faecalis* cells with the feed. Feed samples that received aliquots of double-distilled water served as the control treatment.

Enumeration of *E. faecalis* in poultry and cattle feed, in triplicate samples, was performed following the procedures outlined by Yezerski et al. *(35)*. Poultry or cattle feed (1 g) was added to 9 ml of PBS (pH 7.2; MP Biomedicals) under sterile conditions, shaken, and allowed to settle. One milliliter of the sample was serially diluted in 9 ml of PBS. A 100-μl sample from serial dilutions was drop plated on mEnterococcus agar (Difco) containing tetracycline (40 mg/liter) and incubated at 37°C for 48 h. The *E. faecalis* colonies were counted to determine the concentration of enterococci in poultry and cattle feed. The mean ± standard error (SE) ($n = 3$) initial enterococcal concentrations in poultry feed and in cattle feed were $4.6 \times 10^6 \pm 0.3 \times 10^6$ and $4.7 \times 10^6 \pm 0.3 \times 10^6$ CFU/g, respectively.

Insect exposure to *E. faecalis*–inoculated feed. Inoculated feed samples were placed in sterilized glass petri dishes (60 by 15 mm). In each petri dish 2 g of feed was introduced. Cultures of *T. castaneum* were reared in the Department of Grain Science and Industry, Kansas State University, Manhattan, in environmental chambers (model I-36 VL, Percival Scientific, Perry, IA) on sterile whole wheat flour plus 5% (by weight) brewer's yeast diet at 28°C with 65% relative humidity. Adults (2 weeks old) were separated from the diet by use of an 840-μm-pore-size sieve. Adults separated from the diet were starved for 24 h before use in tests. Before commencing the actual experiments, 10 unsexed adults of mixed ages from laboratory colonies were individually tested for the presence of *E. faecalis* by using serial dilutions and the drop plate technique. Individual insects were homogenized in PBS and drop plated on mEnterococcus agar (Difco) containing tetracycline (40 mg/liter). The plates were allowed to dry and then placed in an incubator at 37°C for 24 to 48 h. After the incubation, the CFU were counted to determine the concentration of enterococci in the insect.

Four separate tests were conducted with poultry and cattle feed to determine the survivability of enterococci in animal feed and to determine the vector competence of *T. castaneum* adults. In the first test, 24 sterile petri dishes, each containing five *T. castaneum* adults with 2 g of poultry feed inoculated with *E. faecalis,* were incubated at 28°C with 65% relative humidity for 7 days. On days 1, 3, 5, and 7 postinfestation, three petri dishes (replicates) were sampled at random to enumerate *E. faecalis* organisms in feed as well as in insects as described above. For enumerating *E. faecalis* in feed, 1 g of the feed was used following the procedures described above. To determine the concentration of *E. faecalis* on the surface and within the gut of *T. castaneum* (non–surface-sterilized insects), two, two, and three adults from replicates 1, 2, and 3, respectively, were selected and *E. faecalis* was isolated from individual insects. The remaining three, three, and two adults from replicates 1, 2, and 3, respectively, were surface sterilized with 10% sodium hypochlorite and 70% ethanol before isolation and enumeration of *E. faecalis*. In the second test, a setup similar to that used for poultry feed was used to determine the concentration of *E. faecalis* in cattle feed on days 1, 3, 5, and 7 and on surface-sterilized and non–surface-sterilized *T. castaneum* adults. In the third and fourth tests, the vector competence of starved (24 h) *T. castaneum* adults in transferring *E. faecalis* from inoculated poultry and cattle feeds to fresh sterilized poultry and cattle feeds, respectively, was assessed. This test for each feed involved two separate sets of 24 sterile petri dishes. Each dish with either poultry or cattle feed was infested with five adults. On alternate days (1, 3, 5, and 7 days postinfestation) three petri dishes were selected at random and the five beetles from each dish were transferred to three new dishes with sterile poultry or cattle feed. After 48 h the sterile poultry feed samples (1 g) were tested for the presence of *E. faecalis* following procedures described above.

Each of the four separate tests had corresponding controls (double-distilled water treatments), and these samples were handled the same way as enterococcus-treated samples.

Experimental design and statistical analysis. A completely random design was used for all experiments. Data on *E. faecalis* CFU (x) in poultry feed over time, in surface-sterilized and non–surface-sterilized *T. castaneum* adults in poultry feed, and transfer of *E. faecalis* by *T. castaneum* adults from inoculated to sterile

TABLE 1. *Concentration of* E. faecalis *in poultry feed, in non–surface-sterilized and surface-sterilized* T. castaneum *adults, and that transmitted by* T. castaneum *to sterile feed*[a]

Time (days)	Concn (mean ± SE) of *E. faecalis*: In poultry feed (CFU/g)	In *T. castaneum* adults (CFU/insect): Non–surface sterilized	In *T. castaneum* adults (CFU/insect): Surface sterilized	Transmitted by *T. castaneum* to sterile feed (CFU/g)
1	$(3.3 \pm 0.3) \times 10^6$ A[b]	$(3.8 \pm 0.6) \times 10^4$ A	$(2.1 \pm 0.1) \times 10^3$ A	$(1.8 \pm 0.1) \times 10^3$ A
3	$(6.7 \pm 0.5) \times 10^4$ B	$(5.1 \pm 0.2) \times 10^3$ B	$(2.8 \pm 0.4) \times 10^3$ A	$(7.6 \pm 0.8) \times 10^2$ A
5	$(2.0 \pm 0.1) \times 10^4$ C	$(2.5 \pm 0.3) \times 10^3$ C	$(1.0 \pm 0.2) \times 10^3$ B	$(2.6 \pm 0.3) \times 10^2$ A
7	$(1.1 \pm 0.1) \times 10^4$ D	$(1.8 \pm 0.1) \times 10^3$ C	$(4.6 \pm 0.6) \times 10^2$ B	$(1.0 \pm 0.5) \times 10^2$ B

[a] The mean initial enterococcal concentration in poultry feed ($n = 3$) was $4.6 \pm 0.3 \times 10^6$ CFU/g; each mean is based on three replicates.
[b] Means within a column followed by different letters are significantly different ($P < 0.05$) by Fisher's protected least significant difference test.

poultry feed were transformed to log(x) scale before statistical analysis to normalize heteroscedastic treatment variances. Similar data for the cattle feed were transformed to log($x + 1$) scale because of zero counts on day 7 in three of the four tests. Significant differences over time of transformed count data for *E. faecalis* in poultry or cattle feed, in surface-sterilized or non–surface-sterilized insects, and in sterile poultry or cattle feed were determined by subjecting data to one-way analysis of variance followed by Fisher's protected least significant difference test with α value set at 0.05 *(29)*.

The logarithmic decrease of *E. faecalis* counts in poultry or cattle feed, in surface-sterilized or non–surface-sterilized insects in poultry and cattle feed, and in sterile poultry or cattle feed over time (days 1 through 7) was characterized by linear regression *(29)*. The inverse of the slope gave a *D*-value, which represented the number of days required for a 1-log reduction of enterococcal concentration *(32)*.

RESULTS

The control feed samples in all tests and the 10 adults tested prior to use in tests were all negative for enterococci. Furthermore, there was no mortality of *T. castaneum* adults exposed to control and enterococcus-treated feed in all of the tests. Therefore, the results only from enterococcus-treated samples are described below.

Survival of *E. faecalis* in feed substrates and insects. The presence of *E. faecalis* in poultry and cattle feeds indicated that the inoculations were successful. The survival of *E. faecalis* decreased significantly in poultry ($F = 1174.85$; df = 3, 8; $P < 0.0001$) (Table 1) and in cattle feed ($F = 319.35$; df = 3, 8; $P < 0.0001$) (Table 2) over the 7-day test period. Adults of *T. castaneum,* allowed to infest the inoculated feed for 7 days, also tested positive for *E. faecalis*. Adult *T. castaneum* in poultry feed acquired *E. faecalis* within 24 h of infestation and retained it during the 7-day test period (Table 1). However, surface-sterilized and non–surface-sterilized *T. castaneum* adults in cattle feed tested negative for *E. faecalis* on day 7 (Table 2).

The *E. faecalis* survival in *T. castaneum* adults fed inoculated poultry feed also decreased significantly in non–surface-sterilized ($F = 393.71$; df = 3, 8; $P < 0.0001$) and surface-sterilized insects ($F = 27.11$; df = 3, 8; $P = 0.0002$). Similarly, *E. faecalis* survival decreased significantly only in non–surface-sterilized *T. castaneum* adults fed inoculated cattle ($F = 469.26$; df = 3, 8; $P < 0.0001$). However, this trend was not apparent in surface-sterilized *T. castaneum* adults ($F = 3.15$; df = 3, 8; $P = 0.0865$).

Vector competence of *T. castaneum* adults. Adults of *T. castaneum* were able to acquire *E. faecalis* and transfer it to sterile poultry feed (Table 1) and cattle feed (Table 2) during the 7-day test period. Adults were unsuccessful in transferring *E. faecalis* to sterile cattle feed on day 7. The number of *E. faecalis* transferred by *T. castaneum* adults to sterile poultry feed showed a significant decrease during the 7-day test period ($F = 4.69$; df = 3, 8; $P = 0.0358$). A

TABLE 2. *Concentration of* E. faecalis *in cattle feed, in non–surface-sterilized and surface-sterilized* T. castaneum *adults, and that transmitted by* T. castaneum *to sterile feed*[a]

Time (days)	Concn (mean ± SE) of *E. faecalis*: In cattle feed (CFU/g)	In *T. castaneum* adults (CFU/insect): Non–surface sterilized	In *T. castaneum* adults (CFU/insect): Surface sterilized[c]	Transmitted by *T. castaneum* to sterile feed (CFU/g)
1	$(1.3 \pm 0.3) \times 10^6$ A[b]	$(2.9 \pm 0.1) \times 10^3$ A	$(1.1 \pm 0.1) \times 10^3$	$(3.3 \pm 0.3) \times 10^2$ A
3	$(2.5 \pm 0.2) \times 10^4$ B	$(1.1 \pm 0.6) \times 10^3$ B	$(3.3 \pm 0.1) \times 10^2$	$(1.6 \pm 0.6) \times 10^2$ A
5	$(1.2 \pm 0.1) \times 10^4$ B	$(4.0 \pm 0.1) \times 10^2$ C	$(2.0 \pm 0.1) \times 10^2$	$(3.3 \pm 0.3) \times 10^1$ B
7	$(2.0 \pm 0.3) \times 10^3$ C	0 D	0	0 C

[a] The mean initial enterococcal concentration in cattle feed ($n = 3$) was $4.7 \pm 0.3 \times 10^6$ CFU/g; each mean is based on three replicates.
[b] Means within a column followed by different letters are significantly different ($P < 0.05$) by Fisher's protected least significant test.
[c] Means among days are not significantly different from one another ($F = 3.15$; df = 3, 8; $P = 0.0865$ [one-way analysis of variance]).

similar trend was evident in tests with the sterile cattle feed ($F = 12.16$; df = 3, 8; $P = 0.0024$).

***D*-values for *E. faecalis* survival in feed substrates and insects.** The relationship between the logarithmic reduction of *E. faecalis* concentration in the feed and in non–surface-sterilized and sterilized insects and in sterile feed and time was satisfactorily described by linear regressions (Table 3). The r^2 values ranged from 0.80 to 0.99. The reduction of *E. faecalis* concentration was more rapid in cattle feed than in poultry feed as indicated by the steeper slope values in cattle than in poultry feed. Similarly, the *E. faecalis* concentration in insects fed cattle feed when compared with that in insects fed poultry feed showed a similar trend. On sterile cattle feed, the *E. faecalis* concentration also decreased more rapidly than in sterile poultry feed. The *D*-values revealed that a 1-log reduction of *E. faecalis* concentration in cattle feed required 2.43 days whereas in poultry feed it was slightly longer (2.60 days). The non–surface-sterilized and surface-sterilized insects in poultry feed had *D*-values that ranged from 4.80 to 8.30 days, whereas corresponding values for insects in cattle feed ranged from 1.85 to 2.63 days. The *D*-value for enterococci in sterile poultry feed was 3.14 days, whereas the comparable value for cattle feed was 2.20 days.

DISCUSSION

Previous work by the authors *(2)* showed the mean ± SE enterococcal concentration in animal feed to be $4.1 \times 10^1 \pm 2.3 \times 10^3$ CFU/g. In adult *T. castaneum* collected from feed manufacturing facilities, the mean ± SE enterococcal concentration was $4.1 \times 10^1 \pm 0.1 \times 10^1$ CFU per insect. A high concentration of *E. faecalis* was used in the present study to ensure survival of enterococci in the feed substrates and to increase the probability of acquisition of *E. faecalis* by *T. castaneum* and its transmission to sterile feed.

In this investigation, *E. faecalis* survived longer in poultry feed than in cattle feed. This likely reflects the fact that the type of animal and plant origin by-products used in feed plays an important role in bacterial contamination and survival in animal feed *(21, 27)*. For example, poultry feed used in this study contained fish meal and amino acids (methionine and L-lysine), which were absent in cattle feed. The presence of salt (NaCl) in poultry feed can make a difference by contributing to the competitiveness of the bacteria under adverse environmental conditions *(26)*. The poultry feed pH is around 6.5, which is within the tolerance range (pH 4.5 to 10) of enterococci *(8)*. All these factors may have contributed to better survival of *E. faecalis* in poultry feed compared with cattle feed. However, the exact role and mechanism are not known and warrant further study.

The low enterococcal loads observed in feed and in insects in our previous studies *(2)* are probably due to the rapid decrease of enterococci as observed in this study. It is important to understand the factors that promote survival of enterococci in both feed and insects. Temporal variation in enterococcal concentration in both feed and insects is

TABLE 3. *Regression estimates showing logarithmic reduction in* E. faecalis *counts in feed substrates and in non–surface-sterilized and surface-sterilized* T. castaneum *adults*[a]

Parameter	*T. castaneum* adults in:							
	Inoculated feed		Poultry feed		Cattle feed		Sterile feed	
	Poultry	Cattle	Non–surface sterilized	Surface sterilized	Non–surface sterilized	Surface sterilized	Poultry	Cattle
Intercept	6.50 ± 0.54	6.10 ± 0.46	4.60 ± 0.30	3.60 ± 0.20	4.40 ± 0.84	3.50 ± 0.12	3.60 ± 0.22	3.10 ± 0.33
Slope	−0.40 ± 0.12	−0.41 ± 0.20	−0.20 ± 0.06	−0.10 ± 0.04	−0.54 ± 0.20	−0.40 ± 0.02	−0.30 ± 0.05	−0.45 ± 0.10
r^2	0.84	0.90	0.85	0.80	0.80	0.99	0.94	0.94
D-value (days)[b]	2.60	2.43	4.80	8.30	1.85	2.63	3.44	2.20

[a] Values are means ± SE.
[b] The *D*-value shows 1-log reduction in *E. faecalis* concentration as a function of time in days.

needed to understand the importance of enterococci in feed environments and their spread by stored-product insects.

Adults of *T. castaneum* are commonly associated with feed mills *(10, 17, 19, 33)*. Adults of *T. castaneum* successfully acquired, retained, and transmitted *E. faecalis* from inoculated feed to sterile feed. This suggested survival of *E. faecalis* for at least a short time in the digestive tract of adult *T. castaneum,* which has a pH of 5.6 to 7.0 *(34)*, conducive for survival of enterococci. The digestive tract of adult insects provides an ideal microclimate for bacterial growth and development *(7, 23)*, and several authors have also reported acquisition, retention, and transmission of bacterial pathogens in adult stored-product insects *(3, 5, 13, 14, 18, 31)*.

The enterococcal concentration in non–surface-sterilized *T. castaneum* adults was generally higher than in surface-sterilized adults, irrespective of the feed, indicating that *E. faecalis* was present on the body surface. Interestingly, the *D*-values were higher for *E. faecalis* from surface-sterilized insects on both feeds than for those from non–surface-sterilized insects. For example, in tests with sterile poultry feed, the *D*-value was 8.30 days for surface-sterilized insects whereas it was 4.80 days for non–surface-sterilized insects. The rapid loss of *E. faecalis* on the surface of the insects in non–surface-sterilized insects compared with surface-sterilized insects may be due to competition from other bacteria on the insect's cuticle.

Our results show that poultry and cattle feed support *E. faecalis* infection but the inoculum tends to decrease at a logarithmic rate over time. It is during these short time periods that *E. faecalis* can be potentially acquired and transmitted to fresh feed by *T. castaneum* adults. The perception that stored-product insects are just aesthetic contaminants is no longer tenable, because these adults can serve as potential vectors of antibiotic-resistant enterococci within the feed manufacturing environment. Therefore, it is important to follow proper pest management practices to reduce potential insect vectors in feeds and in the feed manufacturing environments.

ACKNOWLEDGMENT

We thank Adam Fahrenholz, Manager of Kansas State University's pilot feed mill, Department of Grain Science and Industry, Kansas State University, Manhattan, for providing fresh formulated poultry and cattle feed.

REFERENCES

1. Bates, C., K. L. Hiett, and N. J. Stern. 2004. Relationship of *Campylobacter* isolated from poultry and from darkling beetles in New Zealand. *Avian Dis.* 48:138–147.
2. Channaiah, L. 2009. Polyphasic characterization of antibiotic resistant and potentially virulent enterococci isolated from animal feed and stored-product insects. Ph.D. thesis. Kansas State University, Manhattan.
3. Crumrine, M. H., V. D. Foltz, and J. O. Harris. 1971. Transmission of *Salmonella* Montevideo in wheat by stored-product insects. *Appl. Microbiol.* 22:578–580.
4. Da Costa, P. M., M. Oliveira, A. Bica, P. Vaz-Pires, and F. Bernardo. 2007. Antimicrobial resistance in *Enterococcus* spp. and *Escherichia coli* isolated from poultry feed and feed ingredients. *Vet. Microbiol.* 120:122–131.
5. De Las Casas, E., P. K. Harein, and B. S. Pomeroy. 1972. Bacteria and fungi within the mealworm collected from poultry brooder houses. *Environ. Entomol.* 1:27–30.
6. Devriese, L. A., M. D. Collins, and R. Wirth. 1992. The genus *Enterococcus*, p. 1465–1481. *In* A. Balows, H. G. Traper, M. Dworkin, W. Harder, and K.-H. Schleifer (ed.), The prokaryotes: a handbook on the biology of bacteria, vol. I through IV. Springer-Verlag, New York.
7. Dillon, R. J., and V. M. Dillon. 2004. The gut bacteria of insects: nonpathogenic interactions. *Annu. Rev. Entomol.* 49:71–92.
8. Fisher, F., and C. Phillips. 2009. The ecology, epidemiology and virulence of *Enterococcus*. *Microbiology* 155:1749–1757.
9. Franz, C. M., W. H. Holzapfel, and M. E. Stiles. 1999. Enterococci at the crossroads of food safety? *Int. J. Food Microbiol.* 47:1–24.
10. Hagstrum, D. W., and B. Subramanyam. 2006. Fundamentals of stored-product entomology. American Association of Cereal Chemists International, St. Paul, MN.
11. Harein, P. K., and E. De Las Casas. 1968. Bacteria from granary weevils collected from laboratory colonies and field infestations. *J. Econ. Entomol.* 61:1719–1720.
12. Harein, P. K., E. De Las Casas, C. T. Larsen, and B. S. Pomeroy. 1972. Microbial relationship between the lesser mealworm and its associated environment in a turkey brooder house. *Environ. Entomol.* 1:189–194.
13. Harein, P. K., E. De Las Casas, B. S. Pomeroy, and M. D. York. 1970. *Salmonella* spp. and serotypes of *Escherichia coli* isolated from the lesser mealworm collected in poultry brooder houses. *J. Econ. Entomol.* 63:80–82.
14. Husted, S. R., R. B. Mills, V. D. Foltz, and M. F. Crumrine. 1969. Transmission of *Salmonella* Montevideo from contaminated to clean wheat by the rice weevil. *J. Econ. Entomol.* 62:1489–1491.
15. Krytenburg, D. S., D. D. Hancock, D. H. Rice, T. E. Besser, C. C. Gay, and J. M. Gay. 1998. A pilot survey of *Salmonella enterica* contamination of cattle feeds in the Pacific Northwestern USA. *Anim. Feed Sci. Technol.* 75:75–79.
16. Lambkin, T. A. 2001. A report for the Rural Industries Research and Development Corporation. Publication no. 01/151. RIRDC, Barton, ACT, Australia.
17. Larson, Z., B. Subramanyam, L. Zurek, and T. Hermann. 2008. Stored-product insects associated with eight feed mills in the Midwestern United States. *J. Econ. Entomol.* 101:998–1005.
18. Larson, Z., B. Subramanyam, L. Zurek, and T. Hermann. 2008. Diversity and antibiotic resistance of enterococci associated with stored-product insects collected from feed mills. *J. Stored Prod. Res.* 44:198–203.
19. Loschiavo, S. R., and G. T. Okumura. 1979. A survey of stored product insects in Hawaii. *Proc. Hawaiian Entomol. Soc.* 13:95–118.
20. Lynn, T. V., D. D. Hancock, T. E. Besser, J. H. Harrison, D. H. Rice, and N. T. Stewart. 1998. The occurrence and replication of *Escherichia coli* in cattle feeds. *J. Dairy Sci.* 81:1102–1108.
21. Maciorowski, K. G., P. Herrera, F. T. Jones, S. D. Pillai, and S. C. Ricke. 2007. Effects on poultry and livestock of feed contamination with bacteria and fungi. *Feed Sci. Technol.* 133:109–136.
22. Maciorowski, K. G., P. Herrera, M. M. Kundinger, and S. C. Ricke. 2006. Animal feed production and contamination by foodborne *Salmonella*. *J. Cons. Prot. Food Saf.* 1:197–209.
23. Martin, J. D., and J. O. Mundt. 1972. Enterococci in insects. *Appl. Microbiol.* 21:572–580.
24. Mills, J. T., and N. D. G. White. 1993. Seasonal occurrence of insects and mites in a Manitoba feed mill. *Proc. Entomol. Soc. Manit.* 49:1–15.
25. Myint, M., Y. Johnson, J. Paige, and D. Bautista. 2007. A cross-sectional study of bacterial contamination in plant-protein feed from feed stores in Northern Virginia and Maryland. *Anim. Feed Sci. Technol.* 133:137–148.
26. Neysens, P., W. Messens, and De L. Devuyst. 2003. Effect of sodium chloride on growth and bacteriocin production by *Lactobacillus amylovorus* DCE 471. *Int. J. Food Microbiol.* 88:29–39.
27. Sapkota, A. R., L. Y. Lefferts, S. Mckenzie, and P. Walker. 2007. What do we feed to food-production animals? A review of animal

feed ingredients and their potential impacts on human health. *Environ. Health Perspect.* 115:663–670.
28. Sargeant, J. M., M. W. Sanderson, D. D. Griffin, and R. A. Smith. 2004. Factors associated with the presence of *Escherichia coli* O157 in feedlot-cattle water and feed in the Midwestern USA. *Prev. Vet. Med.* 66:207–237.
29. SAS Institute. 1999. SAS procedures guide, version 9.1. SAS Institute, Cary, NC.
30. Schwalbe, R. S., A. C. McIntosh, S. Qaiyumi, J. A. Johnson, and J. G. Morris, Jr. 1999. Isolation of vancomycin-resistant enterococci from animal feed in USA. *Lancet* 353:722.
31. Skov, M. N., A. G. Spencer, B. Hald, L. Petersen, B. Nauerby, B. Carstensen, and M. Madsen. 2004. The role of litter beetles as potential reservoir for *Salmonella enterica* and thermophilic *Campylobacter* spp. between broiler flocks. *Avian Dis.* 48:9–18.
32. Stumbo, C. R. 1973. Thermobacteriology in food processing. Academic Press, New York.
33. Trematerra, P., and A. Sciarretta. 2004. Spatial distribution of some beetles infesting a feed mill with spatio-temporal dynamics of *Oryzaephilus surinamensis*, *Tribolium castaneum* and *Tribolium confusum*. *J. Stored Prod. Res.* 40:363–377.
34. Vinokurov, K. S., E. N. Elpidina, D. P. Zhuzhikov, B. Oppert, D. Kodrik, and F. Sehnal. 2009. Digestive proteolysis organization in two closely related Tenebrionid beetles: red flour beetle (*Tribolium castaneum*) and confused flour beetle (*Tribolium confusum*). *Arch. Insect Biochem. Physiol.* 70:254–279.
35. Yezerski, A., G. Cussatt, D. Glick, and M. Evancho. 2005. The effects of the presence of stored-product pests on the microfauna of a flour community. *J. Appl. Microbiol.* 98:507–515.