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Thermal Inactivation of *Salmonella* Surrogate, *Enterococcus faecium,* in mash broiler feed pelleted in a university pilot feed mill Microbiology and Food Safety Section

Corey Coe [a], Timothy Boltz [b], Elizabeth Rowen [c], Joe Moritz [a], Gary Freshour [a], Md Shafiul Islam Rion [a], Carly Long [a], Lucas Knarr [a], Kristina Bowen [a], Jacek Jaczynski [a], Kristen Matak [a], Annette Freshour [a], Xue Tang [d], Cangliang Shen [a,*]

[a] School of Agriculture and Food System, West Virginia University, Morgantown, WV, 26506, USA
[b] Department of Poultry Science, Mississippi State University, Mississippi State, MS, 39762, USA
[c] Department of Entomology, University of California Riverside, Riverside, CA, 92506, USA
[d] School of Food Science and Technology, Jiangnan University, Wuxi, 214122, China

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ABSTRACT

This study evaluated the thermal inactivation kinetic parameters of a *Salmonella* surrogate *Enterococcus faecium* ***(E. faecium)*** during feed manufacture in a university pilot feed mill setting. A batch of 227 kg mash broiler feed was pelleted after being inoculated with 1,000 mL of nalidixic acid (**NaL**) resistant *E. faecium* (5.4 $\log_{10}$CFU/g) at 70°, 75°, 80°, and 85°C for 0 to 115 s. Bacterial survival cell counts were analyzed by spread plating onto bile esculin agar plus 200 ppm of NaL. Microbial data and thermal kinetic parameters [n=6, Global-Fit and United States Department of Agriculture (USDA)-Integrated-Predictive-Modeling-Program software] were analyzed by R-software (orthogonal polynomial model). Pelleting mash broiler feed at 70°, 75°, 80°, and 85°C decreased ($P < 0.05$) *E. faecium* cell counts by 0.81, 1.18, 1.69, and 1.94 $\log_{10}$ CFU/g after 115 s, respectively. D-values of orthogonal polynomial, Linear with Tail, Weibull models for *E. faecium* at 70°, 75°, 80°, and 85°C were 47.1 to 135.4, 42.1 to 135.2, and 51.4 to 118.8 s, respectively. These results suggest that pelleting at 80 or 85°C reduces *E. faecium* populations the fastest, and it takes at least 50 s to reduce populations by 1 $\log_{10}$ CFU/g at these temperatures. Thermal inactivation for *E. faecium* took longer and required higher temperatures in the feed mill than lab estimates, highlighting the importance of testing thermal inactivation temperatures in the field to ensure proper feed hygiene.

Introduction

Poultry products are regarded as relatively cheap, easy to prepare, and healthy food choices (Ritchie et al., 2023). It is predicted that Americans will eat 53 kg of poultry meat products per capita in 2024 (National Chicken Council, 2024). Annual global feed production is estimated to be over one billion tonnes, with poultry feed accounting for 44% of total feed produced in 2022 (The International Feed Industry Federation, 2024). Microbial safety is a pivotal concern for poultry feed manufacturing as this is the first component of producing safe consumer products.

An increased focus on food safety has recently led to more preventative controls being established to prevent foodborne pathogen outbreaks (Obe et al., 2023). Feed is considered a vector for pathogens in poultry flocks, and *Salmonella* has been identified as one of the most common biological hazards associated with all classes of animal feed (McIlroy, 1996; Jones, 2011). Due to this, an increased focus on feed hygienics is now placed on feed manufacturers to produce pasteurized feed while still meeting the nutritional requirements of the intended animal. Feed manufacturers have the tools needed to meet this goal, but more research is needed to demonstrate the proper time and temperature combinations to achieve adequate reduction in pathogens.

Foodborne pathogens, including *Salmonella* spp., can survive for a long time in various raw materials used for manufacturing animal feed, making it a major microbial safety concern in animal feed (Jones and Richardson, 2004). The lack of uniformity in feed manufacturing

* Corresponding author.
E-mail address: cangliang.shen@mail.wvu.edu (C. Shen).

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between mills, especially during the production of large feed volumes, makes obtaining accurate estimates of feed microbial contamination rates difficult. Recognized strategies that can reduce the likelihood of pathogens contaminating feed belong to 3 categories: 1) efforts to prevent contamination from entering the facility, 2) efforts to reduce microbial multiplication within the facility, and 3) procedures designed to kill the pathogen (Jones, 2011). Reducing pathogens in feed manufacturing facilities involves discovering microbial growth niches, reducing conditions that allow bacterial growth, and killing pathogens through thermal processing by pelleting (Jones, 2011). In 2011, the Food Safety Modernization Act (**FSMA**)-Preventive Controls for Animal Food was signed into law and aimed to improve and optimize feed hygienics to provide safe feed to animals to produce microbial-safe animal products for consumers. However, the current feed manufacturers have not established effective standard processes and preventive control methods to control foodborne pathogens throughout the pelleting process.

The thermal processing of feed using steam has had minimal research validated on how steam conditioning can control pathogenic bacteria in the feed mill setting. This can be attributed to the feed mill environment being more challenging to control and more dynamic than a lab setting. Due to concern for contamination of non-research feed and other biosafety concerns, using foodborne pathogens, such as *Salmonella*, in feed mills is uncommon. Therefore, the use of surrogate organisms is a viable way to develop Hazard-Analysis-Critical-Control-Point (**HACCP**) plans for feed mills, including identifying the critical control points (**CCPs**) and critical limits (**CLs**) of conditioning temperatures and times. When evaluating the efficacy of given processing treatments in food systems, surrogate microorganisms are commonly used instead of foodborne pathogens in challenge tests conducted in food manufacturing plants (Hu and Gurtler, 2017).

According to the U.S. Food & Drug Administration (FDA), foodborne pathogen surrogate bacteria are defined as non-pathogenic strains responding to a specific treatment similar to a target pathogenic strain (U.S. FDA, 2008). The desired characteristics of an appropriate surrogate are non-pathogenic, inactivation kinetics comparable to the target pathogen, easy to prepare, genetically stable, and susceptible to an injury similar to the pathogen (U.S.-FDA, 2008). For the thermal inactivation treatment, an ideal surrogate should behave similarly or be more resistant to the target pathogen (Hu et al., 2017). Currently, the feed manufacturing industry does not have an industry-wide established preventive control approach to inactivate foodborne pathogens such as Salmonella spp. during the pelleting process. Although Salmonella spp. are not commonly used for validation studies in actual feed mills due to biosafety II level concerns, proposed thermal inactivation methods are still needed and can be done more efficiently in a pilot feed mill setting.

Enterococcus faecium (*E. faecium)* is a recognized surrogate bacterium used in *Salmonella* reduction research, with two common lab strains being American Type Culture Collection (**ATCC**) 8459 and ARS Culture Collection (**NRRL**) B-2354 (Kopit et al., 2014; Ceylan and Bautista, 2015). These strains of *E. faecium* are well documented to be used as they both lack virulence factors known for this species and are sensitive to medically relevant antibiotics (Kopit et al., 2014). Kinetic parameters of *Salmonella* and the surrogate *E. faecium* in mash broiler feed during thermal inactivation in a lab-based thermal dynamic water bath were recently studied in this lab, and the results suggested that *E. faecium* can be used as a surrogate for *Salmonella* to validate thermal inactivation during feed manufacturing (Coe et al., 2022). This preliminary finding allows for modeling the thermal inactivation of *E. faecium* as a surrogate for *Salmonella* in mash broiler feed in a pilot feed mill setting.

To continue to grow the small to large industrial-scale poultry industry in West Virginia, the West Virginia University (**WVU**) Animal Science Research, Education and Outreach Center (REOC) recently renovated its existing feed mill, which enables the training of individuals to understand and be proficient in manufacturing practices, including how feed manufacturing can influence the overall feed microbial load. Because thermal inactivation is a primary mechanism to reduce microbial contamination in feed mills, this study aims to evaluate and model the thermal inactivation of the *Salmonella* surrogate bacteria *E. faecium* in mash broiler feed, specifically in a pilot feed mill setting.

Materials and methods

Feed Manufacture

A corn and soybean meal-based broiler finisher diet was formulated to meet or exceed the Ross 708 nutrient specifications (Aviagen, 2024). Eight, 908 kg masterbatches of feed were mixed before the experiment's start. To mimic the industry practice of adding a portion of oil/fat pre-pelleting, soybean oil was added at a 1% inclusion rate at the mixer. These masterbatches were then allocated to 1 of 33 experimental batches to be used for pelleting. This was done to ensure all experimental treatments contained feed from each master batch to limit any natural bacterial or nutrient concentration variation between the 33 experimental batches.

All feed was pelleted at the WVU pilot feed mill located in Morgantown, West Virginia, during May 2024 over a 3-day period. Days for pelleting were chosen to have similar ambient temperatures to reduce variation during pelleting. This followed the methodologies of Boltz et al. (2021) and Bowen et al. (2022) to eliminate confounding error during the study. A California Pellet Mill (**CPM**) conditioner, CPM hygieniser, 40 HP pellet mill (Master Model Pellet Mill, CPM, Crawfordsville, IN), and 4.5 × 32mm CPM pellet die was utilized. Steam pressure was throttled to 276 kPa prior to the Masoneilan Valve and entrance to the conditioner. Pellet mill motor load was kept constant between 38 to 44% for all treatments and days of manufacture. For this experiment, the hygieniser was not activated; therefore, no heated feed retention occurred. However, feed still passed through the hygieniser for 45 s before being pelleted; as there is no bypass within this feed manufacture system.

Beginning each day of manufacture, the internal metal components of the WVU pelleting system were warmed by pelleting 227 kg of a high-fiber formulation ruminant diet. The high fiber content heated the steel housing of the mill system and generated friction in the pellet die, without necessitating a high volume of warm-up feed.

Preparation and inoculation of bacterial inoculum

The nalidixic acid (**NaL**) resistant *Salmonella* surrogate *E. faecium* ATCC 8459 was used in this study, and the preparation was according to our previous work from this lab using mash broiler feed in lab-based studies (Boltz et al., 2021; Coe et al., 2022). Single colonies of *E. faecium* strain were cultivated on bile esculin agar (**BEA**) plus 200 ppm of NaL (BEA-NaL, Hardy Diagnostics, Santa Maria, CA, U.S.A) and stored in a refrigerated incubator for 1 week before refreshment. The day before the pilot plant experiment, 2 single colonies of *E. faecium* were picked from BEA-NaL and added into a 1,000 ml of tryptic soy broth (**TSB**; Alpha Biosciences, Baltimore, MD, U..SA) containing 200 ppm of NaL for growing at 35°C for 24 h. A total of 20 bottles were prepared each day for each trial. The initial inoculum level of *E. faecium* in each bottle was ~9.0 log10 CFU/ml, confirmed by spread plating onto BEA plus 200 ppm of NaL. Bacterial inoculum in 2 bottles of 1,000 ml TSB plus NaL was manually added into 227 kg of the feed prior to mixing.

Thermal inactivation of mash broiler feed in a pilot feed mill setting

Each 227 kg batch of feed was added to a 1-ton, vertical screw Easy Automation Inc. Modular Feed Processor (Easy Automation Inc., Welcome, MN), and the 2 bottles of inoculum were added and allowed to mix for 2 minutes before being augured to the surge bin above the pelleting system. While being conveyed to the surge bin, 10 mash feed

samples (zero second time point) were obtained to determine the initial *E. faecium* concentration of the batch of feed before being conditioned in the feed system.

The conditioning time was kept constant for each 227 kg batch of feed, and the conditioning temperature was manipulated. This was done to ensure the correct conditioning time was achieved and to mimic industry settings, as the temperature will likely be manipulated over time once the pelleting process begins. The 11 conditioning times for this experiment were 15, 20, 25, 30, 40, 50, 65, 79, 94, and 115 s, and the 4 conditioning temperatures were 70, 75, 80, and 85°C. Due to the pelleting process, the temperature may increase when the feed is extruded through the pellet die. Based on recent work from the same pilot feed mill, it is expected that a 2°C increase in temperature could occur when the feed was being extruded through the pellet die (Knarr et al, 2024). This temperature increase would be only for a short time, would be consistent across all treatments, and would mimic what would be observed in larger feed mills. The research did monitor for carryover of *E. faecium* in the feed manufacture system. Based on bacterial recovery data, little to no carryover of *E. faecium* was observed.

The research team used a Beta Raven programmable logic control system (PLC, 120V #10113354; Beta Raven Automation Solutions, St. Charles, MO) to control the conditioning temperatures and times during the experiment. All conditioning temperatures were monitored from the PLC screen inside the feed mill, where the temperature inside the conditioner was displayed. All feed conditioning temperatures were recorded from this display to make the work more applicable to industry conditions, as a PLC is where all time and temperature manipulation would be done.

One target conditioning time and temperature were set on the PLC at a time, and the feed was conditioned until the target temperature was achieved. After achieving the target temperature, samples would be collected from the pellet die. Once the desired samples had been taken for that temperature, the temperature was increased to the next target temperature using the PLC. Once all four temperatures had been achieved for the set conditioning time, the system was allowed to cool before the next batch of feed was augured above the pelleting system. The process would resume again with the next conditioning time, starting at the 70°C temperature and working back up to 85°C. An overview of feed inoculation, pelleting, and sample collection can be found in Fig. 1.

Microbiological analyses

Pelleted feed samples were removed from the cooler deck in the feed mill after being cooled. Two 20 grams of each trail were collected from these samples and placed into filtered Whirl-Pac bags, followed by adding 20 mL of refrigerated TSB with 0.1% sodium pyruvate to recover heat-injured cells (Jiang et al., 2021). Samples were then homogenized in a blender (Microbiology International) for 60 s, followed by 10- or 100-fold serial dilution solutions in sterile 0.1% buffered peptone water (**BPW**), and then plated onto BEA-NaL to numerate survivals of *E. faecium*. Plated agars were incubated at 35°C for 48 h to grow *E. faecium* cells. After incubation, colonies were manually counted to determine bacterial survival ($\log_{10}$ CFU/g) with a detection limit of 0.3 $\log_{10}$ CFU/g.

Data analysis and modeling of bacterial survivals

This pilot plant experiment was conducted by a 4 × 11 factorial design with 4 tested pelleting temperatures at 70, 75, 80 and 85°C and 11 time periods including 15, 20, 25, 30, 40, 50, 65, 79, 94, and 115 s. The experiment was repeated three times with three samples per treatment for each replicate. To characterize the relationship between time and thermal inactivation of *E. faecium,* orthogonal polynomial regressions were used for each temperature in R (version 4.4.1, R Core Team, 2024). Because each temperature treatment resulted in a different response curve for *E. faecium*, no orthogonal polynomial model fit all temperature treatments simultaneously; thus, each was fit separately. A Bonferroni correction was used to account for these separate models ($\alpha = 0.0125$). Each model included $\log_{10}$ CFU/g as the response variable and the day of manufacture, weight of feed, and an orthogonal polynomial term of time (s) as predictors. These models were fit using *lm* in base R. Orthogonal polynomial degrees were added to each model until the residuals were homogenous and normally distributed, tested using the package DHARMa (Hartig, 2024) All predictors were initially interacting, but interactions were removed until all interactions included in the model were significant ($\alpha = 0.05$). Significant differences between initial populations on each day of manufacture were tested

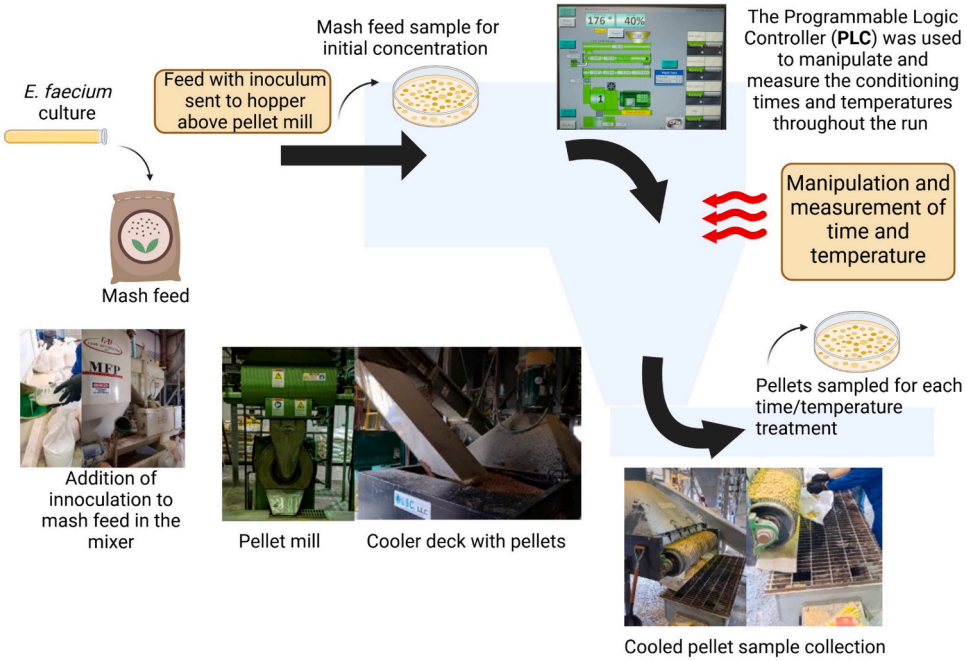

Figure 1. Thermal process outline in the pilot feed mill.

using package emmeans (Lenth, 2024).

These orthogonal polynomial models were used to estimate the mean D-value but also the time (D-value) that it would take to reduce 90% (1-log) of potential bacterial populations. Using the *predict* function in base R, at each temperature, the time it took to reach a colony size of 4.40 $\log_{10}$ CFU/g was estimated for each model as well as the 95% prediction interval around that estimate. This colony size was based on the average starting population (5.4 $\log_{10}$ CFU/g).

Each individual survival data from the four heated temperatures were first analyzed using the USDA-IPMP software (Huang, 2014) which includes 4 bacterial survival models (Linear without and with Tail, Weibull, Re-parameterized Gompertz, and Buchanan Two/Three Phase linear survival models) to determine the fitness of each equation based on the calculated R^2 and Root Mean Square Error (RMSE) values (López-Gálvez et al., 2012; Li et al., 2018). All survival data fit Linear with Tail and Weibull models; therefore, the whole dataset was then modeled using USDA-IPMP-Global fit software (Huang, 2017), which include Weibull model to determine the D-values across all tested samples. Gompertz and Buchanan Two/Three Phase models were not fit for the *E. faecium* data curves.

Results

Physical and chemical characteristics of mash broiler feed

The tested pH, water activity, and moisture content of mash broiler feed samples were 5.70 ± 0.05, 0.705 ± 0.011, and 10.9 ± 0.5%, respectively. After pelleting at 70, 75, 80, and 85°C for 115 s, the moisture content of feed samples decreased to 9.6 ± 0.2, 9.5 ± 0.1, 9.3 ± 0.1, and 9.2 ± 0.1%, respectively. *E. faecium* was not detected in the mash feeds samples used in this study (the detection limit is 0.3 $\log_{10}$ CFU/g).

Survival of surrogate bacteria E. faecium in mash broiler feed after thermal treatments

The bacterial cell survival curve of the *Salmonella* surrogate *E. faecium* cells is shown in Figs 2 and 3. The average initial populations of the surrogate cell counts were 5.35, 5.40, 5.42, and 5.43 $\log_{10}$ CFU/g for samples pelleted at 70°C, 75°C, 80°C, and 85°C, respectively. As expected, the *E. faecium* cells in pelleted feed samples decreased gradually ($P < 0.05$) with increasing retention time in the pelleting process (Figs 2 and 3), and the cell counts decreased at an increased rate with increasing conditioning target temperature (Figs 2 and 3). Pelleting feed at 70°, 75°, 80°, and 85°C decreased ($P < 0.05$) the surrogate cell counts from 5.35-5.40 $\log_{10}$ CFU/g to 4.54, 4.22, 3.73, and 3.49 $\log_{10}$ CFU/g after 115 s, respectively (Fig. 2), with the bacterial cell reductions of 0.79, 1.18, 1.69, and 1.94 $\log_{10}$ CFU/g, as compared to the initial cell counts. The reduction curve of *E. faecium* was flat from 79 to 115 s conditioning at 70 to 85°C (Fig. 2), suggesting a "Tail" effect with a less-heat susceptible subpopulation of *E. faecium* developing in the feed (Fig. 2).

Modeling of thermal inactivation E. faecium in mash broiler feed using a polynomial model from R-software

At 70°C, the best-fit model ($F_{5,60} = 378.6$, $P < 0.0001$, *Adj.* $R^2 = 0.97$, Table 1, Fig. 3) was a first-degree (e.g. linear) polynomial model that included a significant effect on the day of manufacture ($F_{2,61} = 14.6$, $P < 0.0001$), and linear negative effect of time ($F_{1,61} = 498$, $P < 0.0001$), but not the weight of feed ($F_{1,61} = 0.14$, $P = 0.71$). The predicted D-value from this model was beyond the experimental time treatments (135.4 s, 95% PI: 108.0–163.6s, Table 1).

At 75°C, model fit statistics indicated that a fifth-degree orthogonal polynomial was the best fit for the relationship between $\log_{10}$ CFU/g and time; in addition, day of manufacture and the weight of feed ($F_{8,57} = 235.9$, $P < 0.0001$, *Adj.* $R^2 = 0.97$). The orthogonal polynomial of time had a significant effect on *E. faecium* ($F_{5,57} = 374$, $P < 0.0001$), but the day of manufacture ($F_{2,57} = 0.91$, $P = 0.41$) and weight of feed ($F_{1,57} = 1.07$, $P = 0.31$) did not. The predicted D-value from this model was 93.8 s [75.2 – 111.2 95% PI] (Table 1).

The model for 80°C fit with a sixth-degree orthogonal polynomial model ($F_{9,56} = 408.5$, $P < 0.0001$, *Adj.* $R^2 = 0.98$), where the polynomial effect of time influenced *E. faecium* CFUs ($F_{6,56} = 608$, $P < 0.0001$), but, like for 75°C, the day of manufacture ($F_{2,57} = 0.12$, $P = 0.88$) and weight of feed ($F_{1,57} = 0.14$, $P = 0.71$) did not. The predicted D-value from this model was 62.3 s [54.8–68.7s 95% PI] (Table 1).

Finally, the model for 85°C fit with a fourth-degree orthogonal

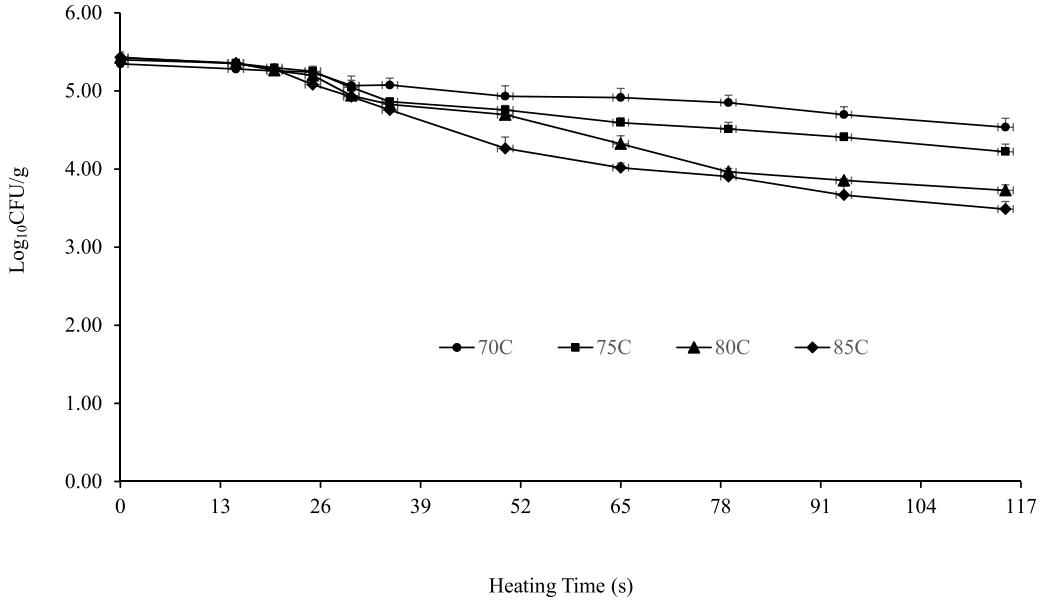

Figure 2. Survival-temperature profiles of the surrogate Enterococcus faecium in broiler mash feed sample heated at 70, 75, 80, and 85°C in in a pilot feed mill setting. Each data point is the average value of 6 replicates.

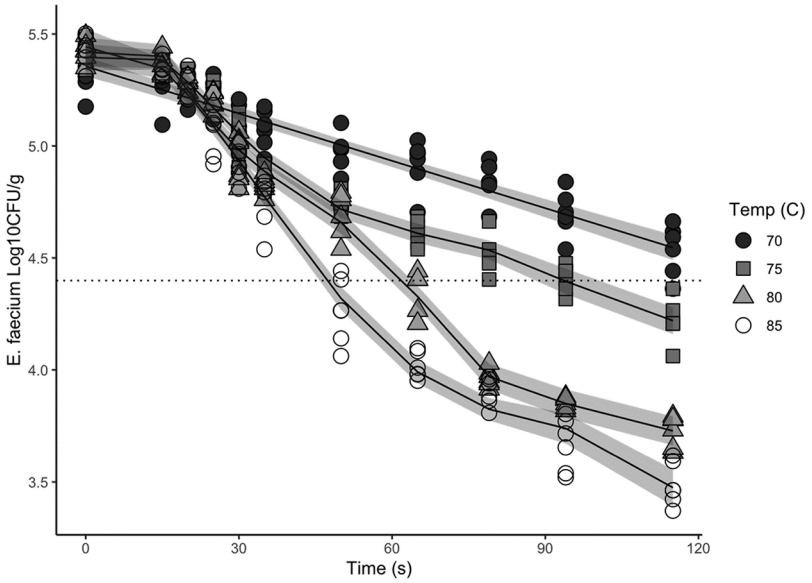

Figure 3. Decrease of *E. faecium* over time (s) in response to temperature treatments at 70, 75, 80, and 85°C in a pilot feed mill setting. Best fit lines are calculated based on individual orthogonal polynomial regression models for each temperature. Gray shading around best-fit lines represents 95% confidence intervals. The dashed horizontal line represents Log_{10} CFU/g = 4.40 used to calculate D-values.

Table 1
Model fit, estimated Y intercept (Y_0), and estimated D-values of *Salmonella* surrogate *Enterococcus faecium* calculated from Orthogonal Polynomial Models (in R).

Temperature (°C)	RMSE	AIC	$Y_0 \pm$ SE	D-value (s) [95%PI]
70	0.084	-127.2	5.35 ± 0.02	135.7* [115.4–161.6]
75	0.068	-147.2	5.40 ± 0.03	93.8 [75.2–111.2]
80	0.074	-134.9	5.41 ± 0.04	62.3 [54.8–68.7]
85	0.094	-106.4	5.44 ± 0.05	47.1 [40.9–49.4]

RMSE-Root Mean Square Error; AIC-Akaike Information Criterion; SE – Standard Error, calculated from model, 95%PI – 95% Prediction Interval.
* beyond the tested range, D-value estimates unreliable

polynomial model ($F_{7,58}$ = 430, $P < 0.0001$, *Adj.* R^2 = 0.98). The polynomial that included time had a strong effect on *E. faecium* CFUs ($F_{4,58}$ = 744, $P < 0.0001$), but the day of manufacture ($F_{2,58}$ = 0.65, P = 0.53) and weight of feed ($F_{1,58}$ = 0.13, P = 0.72) had no effect. The predicted D-value from this model was 47.1 s [40.6– 49.4s 95% PI] (Table 1).

Modeling of thermal inactivation E. faecium in mash broiler feed using USDA-IPMP and USDA-IPMP Global-fit software

Modeling of the thermal inactivation kinetics of *E. faecium* was first done using the USDA-IPMP software (Huang, 2014), including 3 bacterial survival equations Linear, Linear with tail, Gompertz, and Buchanan Two/Three Phase models and determined by the RMSE (close to 0.000) and AIC values (the smaller, the better). As shown in Table 2, Linear with Tail model well fit the survival curves of *E. faecium* across all tested pelleted feed samples. The tested RMSE values ranged from 0.096 to 0.146 (Table 2), and AIC values ranged from -42.79 to -32.16 (Table 2) for Linear with tail model. As expected, the D-values calculated from the "Linear with Tail" model decreased significantly from 135.2 to 42.1 s when the conditioning temperature increased from 70 to 85°C (Table 2). The "Tail" time of 70, 75, 80, and 85°C samples were 4.6, 4.3, 3.8, and 3.6 s, gradually decreasing as the conditioning temperature increased (Table 2).

The IPMP-Global fit software created by Huang (2017), including Weibull model, was used to estimate the D-values of *E. faecium* in feed samples pelleted at 70, 75, 80, and 85°C simultaneously. The D-values of *E. faecium* calculated from Weibull models were 118.8, 84.7, 59.6, and 51.4 s for samples pelleted at 70, 75, 80, and 85°C, respectively, where the k value is 0.950 indicating the concave shape of *E. faecium* survival curve in pelleted feed samples (Table 3).

Table 2
D- and "Tail" values (sec, Mean ± Standard Deviation) of *Salmonella* surrogate *Enterococcus faecium* calculated from Linear with "Tail" models from the USDA Integrated Pathogen Modeling Program for Predictive Microbiology - IPMP software.

Temperature (°C)	RMSE*	AIC*	Y_0	D-value	Tail
70	0.096	-42.79	5.36 ± 0.06	135.2 ± 11.2a	4.6 ± 0.1a
75	0.105	-39.22	5.47 ± 0.04	77.4 ± 10.4b	4.3 ± 0.1b
80	0.112	-37.81	5.57 ± 0.03	52.2 ± 2.0c	3.8 ± 0.1c
85	0.146	-32.16	5.61 ± 0.07	42.1 ± 4.3d	3.6 ± 0.2c

RMSE-Root Mean Square Error; AIC- AIC-Akaike Information Criterion.
Mean values with different letters within a column differ significantly ($P < 0.05$).

Discussion

In this study, the moisture content and water activity values of mash feeds samples decreased after pelleting at 70 to 85°C than the samples before pelleting. As confirmed by previous study in low moisture foods, such in wheat flours (Liu et al., 2018a; 2018b), reduction in moisture content and water activity of the samples significantly increase D values of *Salmonella* and their surrogate *E. faecium*. Liu et al. (2018a) reported that when the moisture content of wheat flour decreased from 14 to 10%, the D-values of *Salmonella* and *E. faecium* at 75 to 85°C increased from 2.7-25.5 to 15.9-65.8 min and 1.1-12.0 to 5.9-24.5 min, respectively. In a related study, Liu et al. (2018b) also found that thermal resistance of *Salmonella* Enteritidis and its surrogate *E. faecium* in wheat flour, as reflected by D values at 80°C, increased from 1.80 to 159.31 min and 3.81 to 281.78 min, respectively, with the water activity values

Table 3
D- and *z*-values (sec, Mean ± Standard Deviation) of *Enterococcus faecium* calculated from Weibull model from the USDA Integrated Pathogen Modeling Program for Predictive Microbiology - IPMP Global-Fit software.

Parameters	*E. faecium*
Weibull Model	
D, T85.0°C	51.4 ± 4.8a
D, T80.0°C	59.6 ± 3.0b
D, T75.0°C	84.7 ± 7.2c
D, T70.0°C	118.8 ± 6.9d
k	0.950 ± 0.058
Y_0	5.47 ± 0.05
RMSE	0.133
AIC	-167.1

RMSE-Root Mean Square Error; AIC- AIC-Akaike Information Criterion. Mean values with different letters within a column differ significantly ($P < 0.05$).

decreasing from 0.70 to 0.11.

E. faecium is a gram-positive, non-endospore forming, facultative aerobic cocci, originally belonging to *Streptococcus* that can survive in various conditions of temperatures, pH, and salt concentrations (Fisher and Phillips, 2009). Previously, *E. faecium* was studied alone during pelleting, and in a side-by-side comparison study with *Salmonella* in our laboratory conditions. Boney et al. (2018) found that conditioning feed for 10 and 60 s achieved a 3- and 4-log reduction of *E. faecium*. Boltz et al., (2019) reported that thermally aggressive pelleting of feed at 80°C for 30 s plus hygieniser retention for 45 s reduced 4-log of *E. faecium*, which was greater than the 3-log reduction from the standard pelleting at 70°C for 15 s without hygieniser use. The recent side-by-side comparison study in a circulated water bath showed that heating *E. faecium* in feed at 75°, 85°, and 95°C for 180, 120, and 70 s achieved reductions of 3, 6, and > 6.5 $\log_{10}$ CFU/g, respectively, which showed significantly more heat resistance than *Salmonella* Typhimurium (Coe et al., 2022). D-values of linear, Weibull models, and z-value of *E. faecium* at 75°, 85°, and 95°C were 3.7 to 18.1, 8.5 to 34.4, and 34.1 s, respectively, which were significantly greater ($P < 0.05$) than those of *Salmonella* Typhimurium (1.8 to 11.2, 4.2 to 21.8, and 28.6 s, Coe et al., 2022). These results clearly suggest that *E. faecium* can be used as a surrogate for *Salmonella* for validation and modeling thermal inactivation kinetics in feed.

The goal of this study was to identify the amount of time required to reduce *E. faecium* as a surrogate for *Salmonella* at different conditioning temperatures in a pilot feed mill. Previous work (Bianchini et al., 2014; Jiang et al., 2021; Coe et al., 2022) has indicated that even low temperatures can reduce *E. faecium* by 3-6 $\log_{10}$ CFU/g within 3 mins. In this pilot feed mill study, pelleting feed at 70 to 85°C for 115 s reduced the surrogate *E. faecium* by only 0.8 to 1.9 $\log_{10}$ CFU/g, and this reduction is less than the previous lab studies of *E. faecium* in food products (Bianchini et al., 2014; Jiang et al, 2021) and feed (Coe et al, 2022). Logically, feed mills, even small feed mills, will require longer conditioning or higher conditioning temperatures to reduce bacterial populations, given the volume and mass of feed relative to laboratory-scale studies. It has been shown that in some industries, the operational and thermal treatment conditions are much less controlled and more variable in the industry scale processing than under university laboratory conditions (Li et al., 2020), and temperature needs time to penetrate and heat the inner mass of feed to get bacterial reduction.

"D-value" is a typical index to evaluate the thermal inactivation kinetics of a microorganism in food products. "D-value" is defined as the time required to inactivate 1-log/unit of the target microorganism in a specific food product at a fixed heating temperature (Jay, 2005). We estimated D-values from our data in multiple ways, including the industry standards (Linear with "Tail" and Weibull models) and using orthogonal polynomial models. Calculating the D-values using multiple methods allows for comparison with other studies, but also allows for models that fit the data tightly. The orthogonal polynomial model fit the data particularly well, with much lower AIC values than the linear with tail and Weibull models, but model parameter estimates are harder to interpret. Regardless of how they were calculated, the D-values calculated from the orthogonal polynomial model (47.1 to 135.4 s) and Weibull models (51.4 to 118.8 s) are greater than our previous studies of D-values of *E. faecium* in mash broiler feed of 3.7 to 18.1 s when heating at 95° to 75°C. The difference in the calculated D-values between the current study and our previous study by Coe et al. (2022) could be due to the mass of the mash feed heated at 75° to 95°C in a laboratory-circulated water bath, compared to the large volume of 227 kg mash feed being processed in the current study. Studies by Bianchini et al. (2014), and Ceylan and Bautista (2015) both concluded that the heat resistance of *E. faecium* could be explained by a sigma factor-mediated adaptation system that was generated during the thermal process to direct its RNA polymerase to transcribe and translate genes to produce heat resistance proteins. As mentioned early, the higher D-values calculated in this study could be due to the reduction in moisture content and water activity of the feed's samples pelleting at 70 to 85°C. Previous studies in low moisture foods, including soy proteins, almond, pistachio, and pecans (Dhowlaghar and Zhu, 2022), peanut butters (Park et al., 2021), chocolates chip cookies (Suhalim et al., 2023) confirmed this theory. For example, in a study of peanut butters, Park et al. (2021) reported that when the water activity values of super-steam heated peanut butter decreased from 0.80 to 0.18, the D-values of *E. faecium* at 125 to 225°C increased from 8.13 to 125 s, 2.98 to 29.94 s, and 0.58 to 21.88 s, respectively. The similar study on chocolate chip cookies also found that the D-values of *E. faecium* heated at 75, 80 and 85°C increased from 9.34 to 13.85 min, 3.01 to 4.94 min, and 1.01 to 1.82 min when moisture content decreased as 17.10, to 12.10, and to 7.86%, respectively (Suhalim et al., 2023).

In conclusion, results from this study suggest that thermal processing of mash broiler feed in a university pilot feed mill at 70° to 85°C after 115 s reduced 0.9 to 2.0 log $_{10}$ CFU/g reduction of the *Salmonella* surrogate *E. faecium*, and the thermal dynamic kinetics well fit the Linear with "Tail" and Weibull models. The authors are aware that the data obtained in this study will only apply to the specific moisture content, process condition, and feed composition, far from being adequate for developing general standard commercial operation procedures in a a standard commercial feed mill for feed manufacturing.

Declaration of competing interest

No conflict

Acknowledgments

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