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REPORT

Determination of the antibacterial properties of wood pellet fines bedding with and without various lime treatments when inoculated with a selection of mastitis and environmental stress resistant related organisms.

Aims

To determine the level of reduction of a selection of mastitis and environmentally stress resistant related organisms when inoculated into wood pellet fines bedding with and without 2 levels of lime designed for use as a disinfectant in livestock stalls.

SUMMARY

Requirements- AFBI in conjunction with Miller Bedding sourced and blended a representative, homogeneous sample of wood pellet fines for use throughout the trial. Three commercially available bedding lime materials provided by Millers were add to the wood pellet fines at 10% and 20% concentrations. These bedding mixtures with and without lime added were inoculated with a cocktail of 5 *E.coli* and 5 *Staphylococcus aureus* strains associated with skin conditions and resistance to environmental stresses. The bedding was tested after 24 and 72 hours to determine the effect (if any) on the levels of the inoculated organisms.

1. Determination of Naturally Occurring Flora

The homogeneous sample of wood pellet fines, a virgin sawdust sample and three samples of bedding from competitors were tested for the presence of naturally occurring Staph. Aureus and E.coli.

2. Preparation of Inoculum

The 5 *E.coli* and 5 *Staphylococcus aureus* strains were grown separately in an enrichment medium appropriate to maximise growth for 24 hours to stationary phase. All 10 strains were combined and harvested by centrifugation. The cells were washed twice in phosphate-buffered saline (PBS). The harvested cells were resuspended in 500ml of PBS and the level of *E.coli* and *Staph. aureus* determined by plate count on the appropriate selective media; Baird Parker Agar for *Staph. Aureus* and TBX agar for *Escherichia coli*.

Baird Parker were incubated at 37ºC for 48 h.

TBX agar plates were incubated at 37ºC for 24 h.

3. METHODS

INOCULATION TRIAL WITH PATHOGENS

To determine the effect of the bedding mixtures on the organisms associated with faeces and skin disorders. Twenty five gram samples of the various bedding/lime combinations were thoroughly mixed with high numbers of the two pathogens; *Escherichia coli* and *Staph aureus* in a 10ml inoculum of washed and resuspended cells.

Strains Used

For *Staphylococcus aureus / epidermidis*, a cocktail containing equal quantities of the following five strains was used to inoculate the bedding formulations:

NCTC 8325

NCTC 7485

NCTC 10655

NCTC 6571

NCTC 11047

(One of these strains is associated with mastitis, two strains are from other skin related infections and the other two are relatively resistant to environmental stresses)

For *Escherichia coli*, a cocktail containing equal quantities of the following five strains was used to inoculate the bedding formulations:

NCTC 11601
NCTC 11602
NCTC 11603
NCTC 9706
NCTC 9707

(All of these strains are from faeces and have been chosen for their additional resistance to environmental stresses)

Inoculation of the wood pellet fines

The bacterial inoculum (10ml) was used to inoculate each of 3 replicate samples of the bedding combinations (25g). The inoculated bedding samples were incubated at 37ºC in sealed bags to retain moisture. The levels of the inoculated organisms were determined by plate counts after 24h and 72h. The level of inoculum with respect to *E.coli* and *Staph aureus* in each bedding sample is given in Table 1 below.

Determination of pH of the Bedding Formulations

Three 25g replicate samples of all of the bedding combinations were prepared and 50ml of de-ionised water added to form a slurry mixture. These were allowed to stand for 30 minutes and the pH measured. The mean pH result for each bedding combinations is given in Table 2 below.

INOCULATION TRIAL RESULTS

1. All bedding samples were tested prior to inoculation for the presence of naturally occurring *E.coli* and *Staph aureus. E.coli* and *Staph aureus* were not detected.

Table 1. Mean Inoculum level of Organisms added to Bedding.

Mean results for 3 replicates:

	Counts for inoculation (Log_{10}CFU/ml)
Staph. aureus	8.25 or 1.8×10^8
Escherichia coli	8.01 or 1.2×10^8

Table 2. Mean pH of Bedding Formulations.

Mean results for 3 replicates:

	pH
100% [redacted] Fines	4.3
100% Sawdust	4.4
10% [redacted] + Fines	10.1
20% [redacted] + Fines	10.1
10% [redacted] + Fines	12.1
20% [redacted] + Fines	12.3
10% [redacted] + Fines	6.6
20% [redacted] + Fines	6.5
[redacted]	4.5
[redacted]	7.7
[redacted]	7.6

Results of Inoculation Trial After 24h incubation-

Mean results for 3 replicates:

	Counts for Staph. aureus (Log_{10}CFU/ml)	Counts for E. coli (Log_{10}CFU/ml)
100% [redacted] Fines	0	0
100% Sawdust	0	0
10% [redacted] + Fines	3.00	7.61
20% [redacted] + Fines	2.48	7.30
10% [redacted] + Fines	0	0
20% [redacted] + Fines	0	0
10% [redacted] + Fines	3.60	6.98
20% [redacted] + Fines	4.11	7.00
[redacted] Mix	0	0
[redacted] Mix	4.00	7.97
[redacted]	4.00	8.10

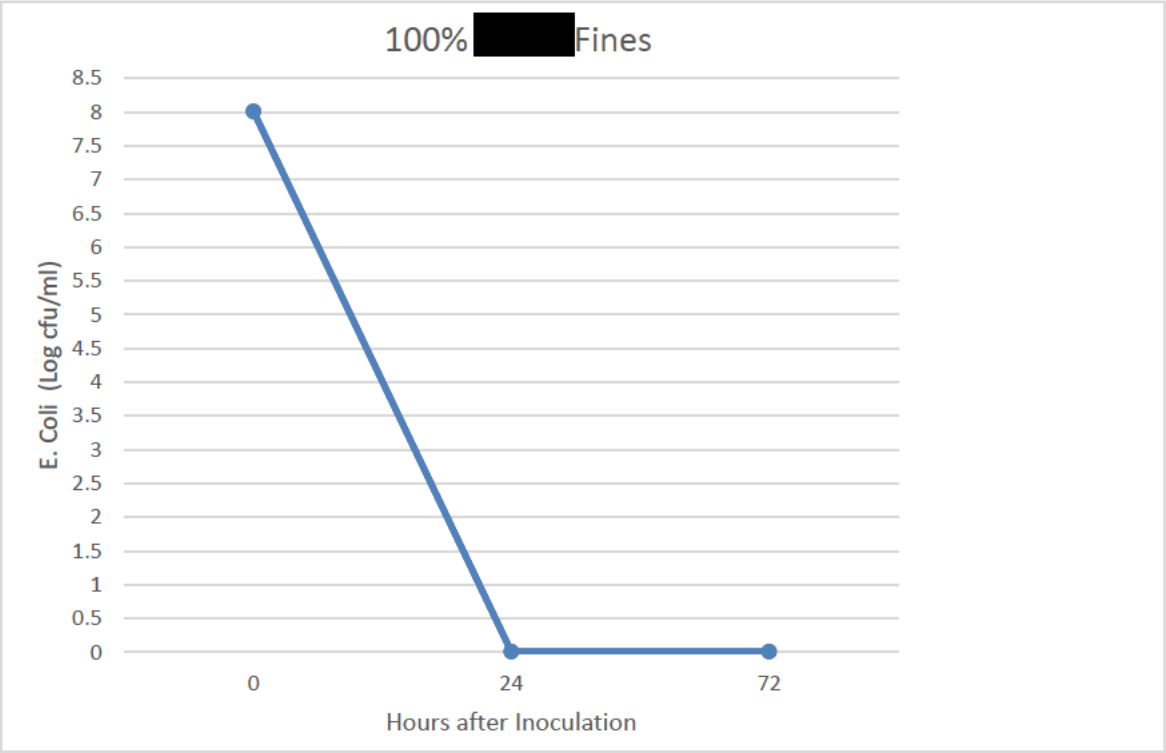
Results of Inoculation Trial After 72h incubation-

Mean results for 3 replicates:

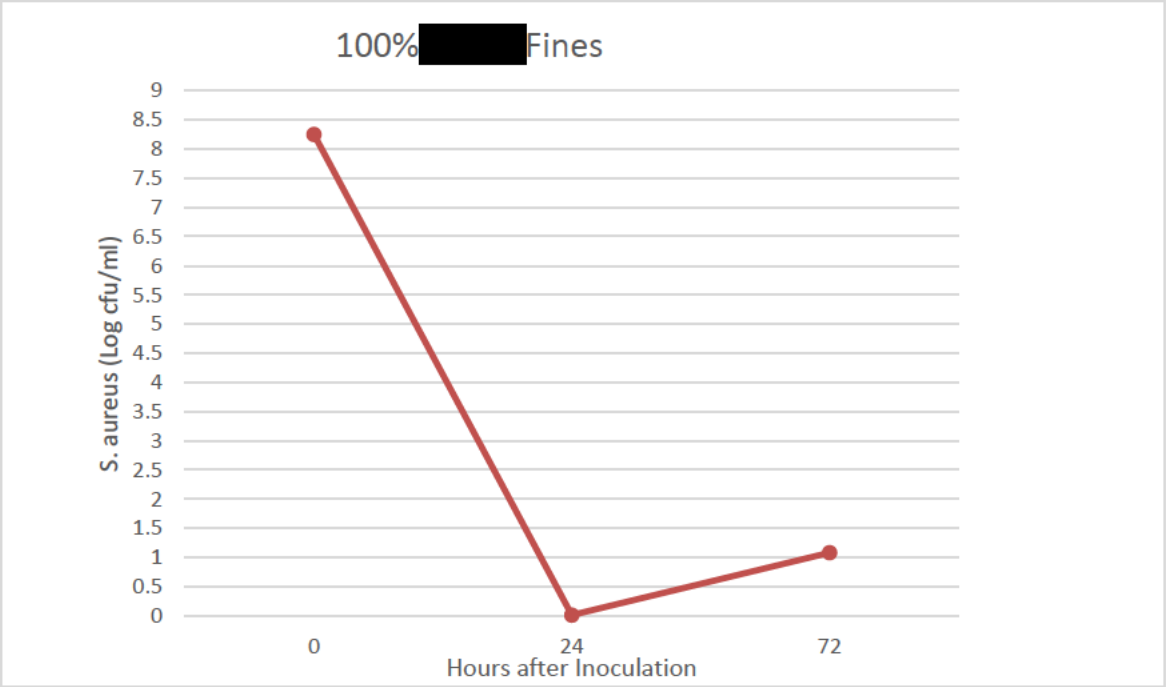
	Counts for Staph. aureus (Log_{10}CFU/ml)	Counts for E. coli (Log_{10}CFU/ml)
100% [redacted] Fines	1.08	0
100% Sawdust	1.00	0
10% [redacted] + Fines	3.99	7.62
20% [redacted] + Fines	3.36	7.60
10% [redacted] + Fines	1.18	4.45
20% [redacted] + Fines	0	0
10% [redacted] + Fines	0	6.20
20% [redacted] + Fines	1.30	8.00
[redacted]	0	0
[redacted]	4.00	7.97
[redacted]	4.00	8.10

RESULTS for [redacted] FINES

For *E.coli*

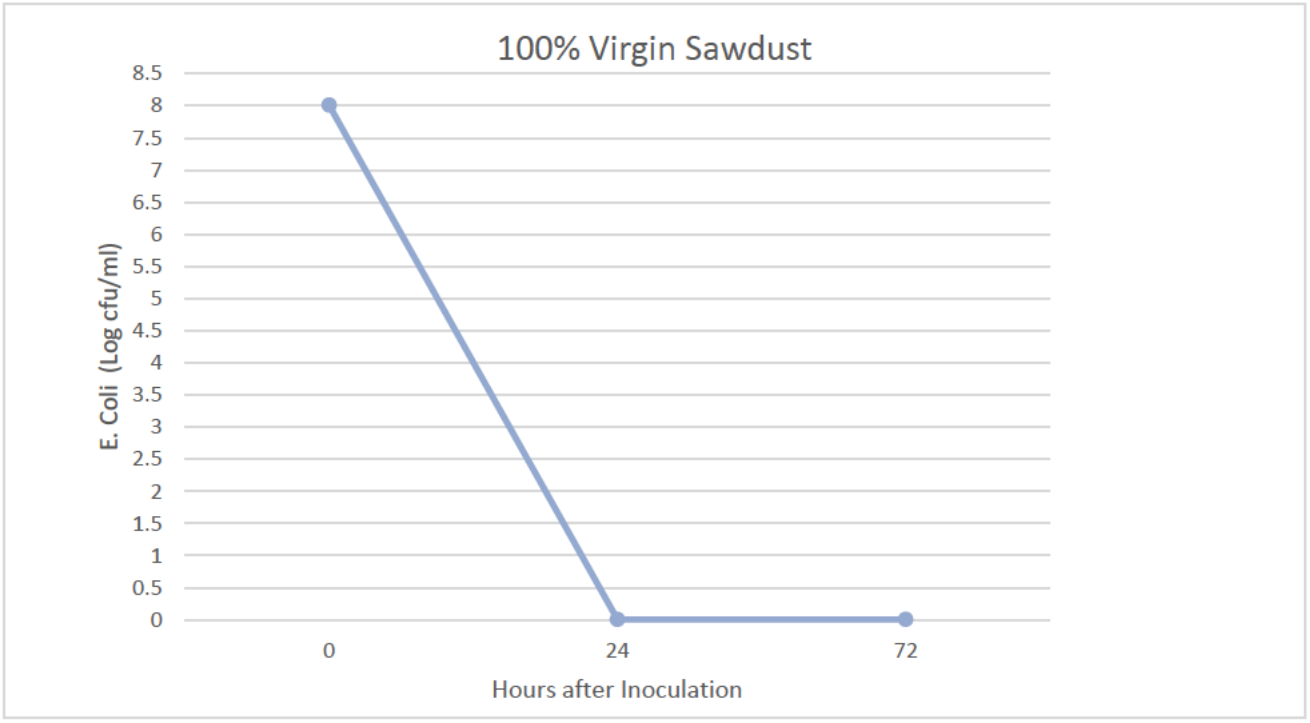


For Staph aureus



RESULTS for SAWDUST

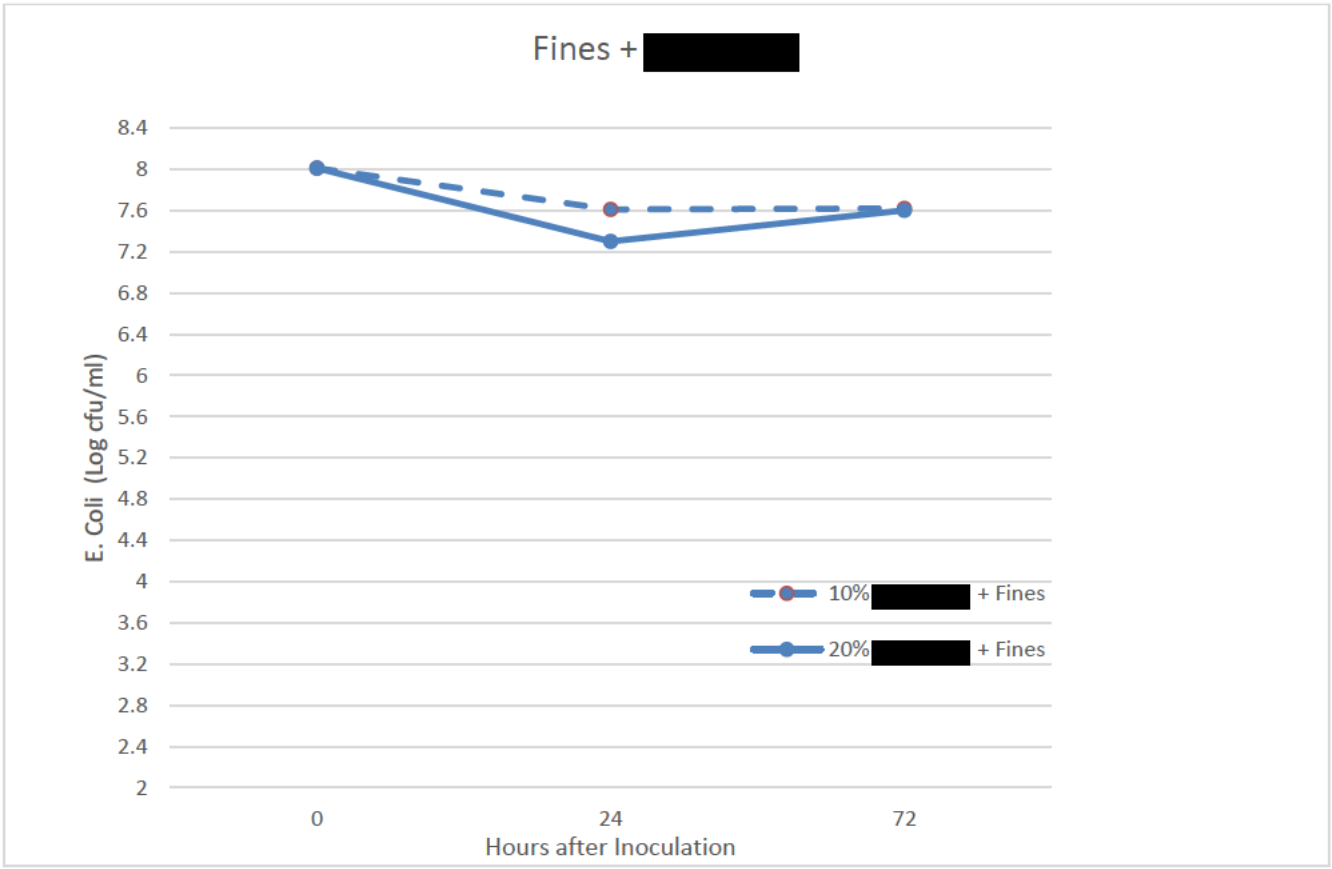
For *E.coli*



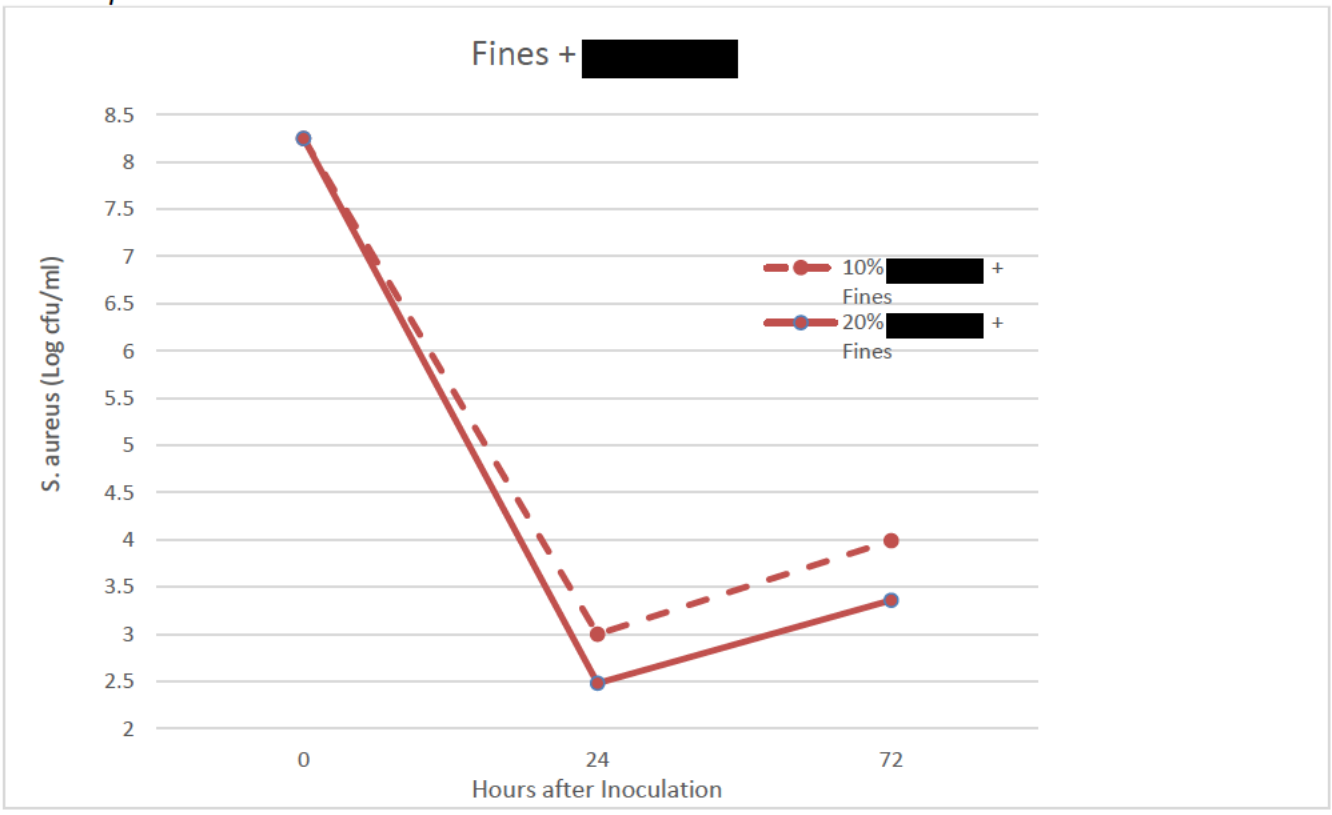
For Staph aureus

RESULTS for FINES + [redacted]

For *E.coli*

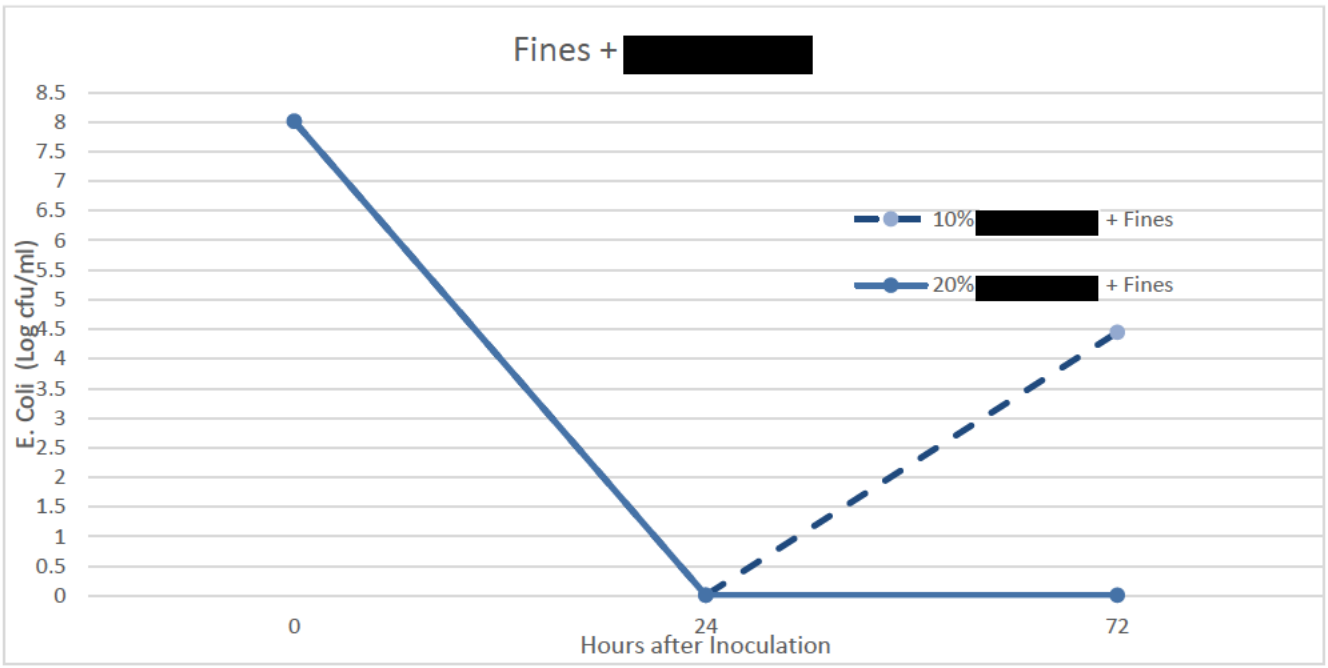


For Staph aureus

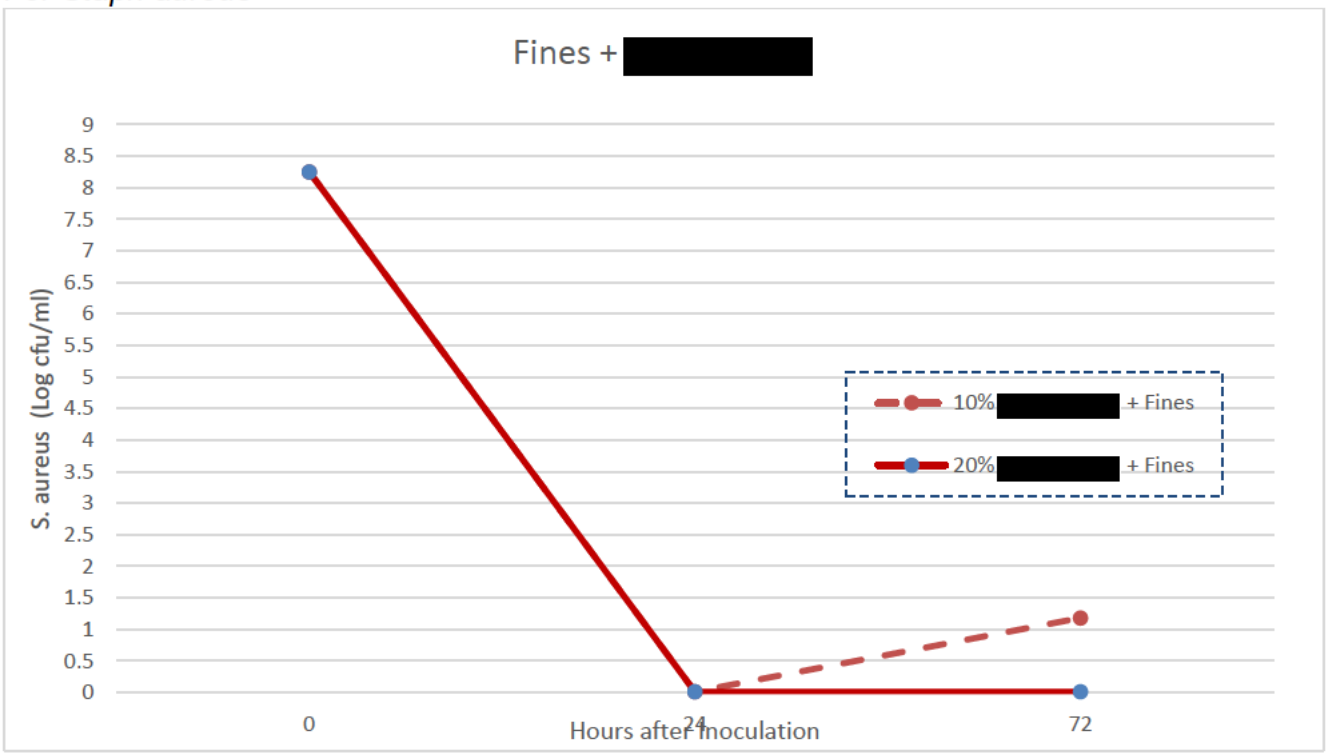


RESULTS for FINES + [redacted]

For *E.coli*

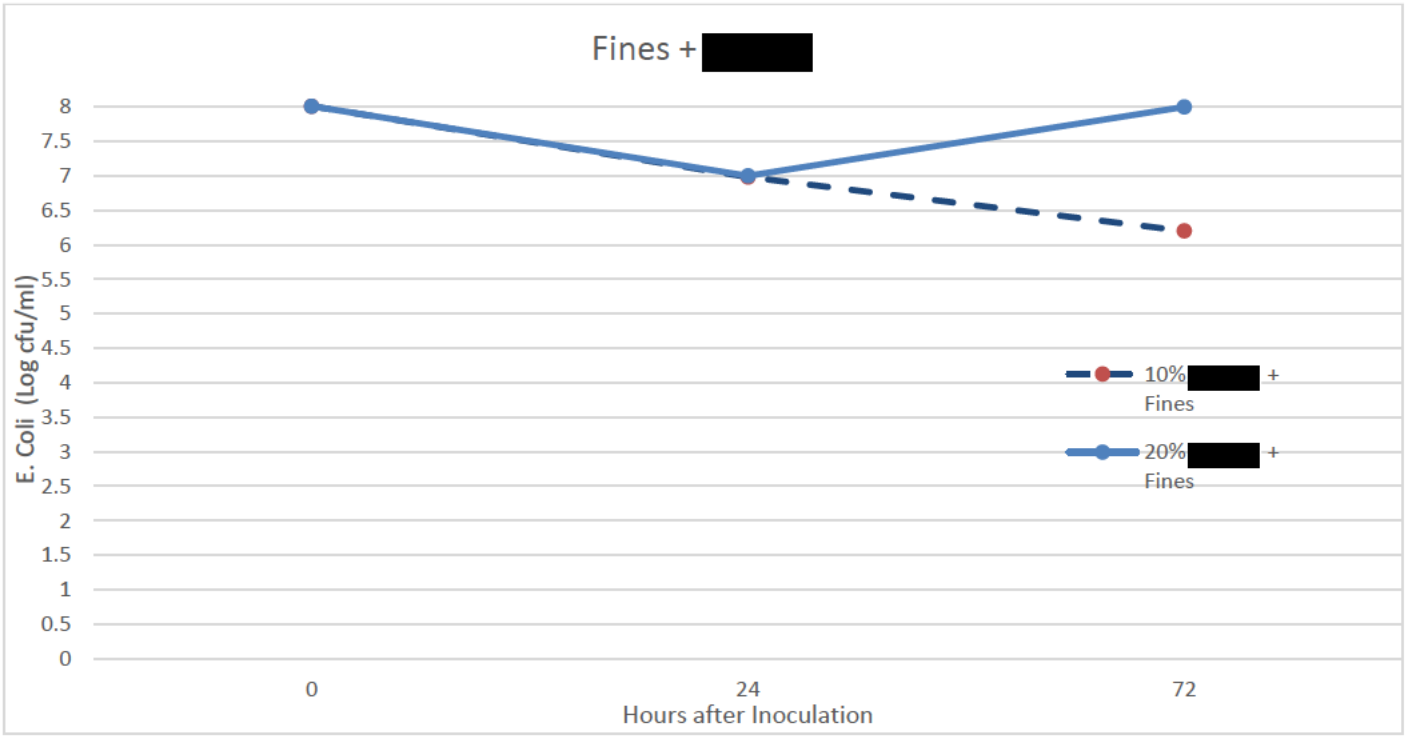


For Staph aureus

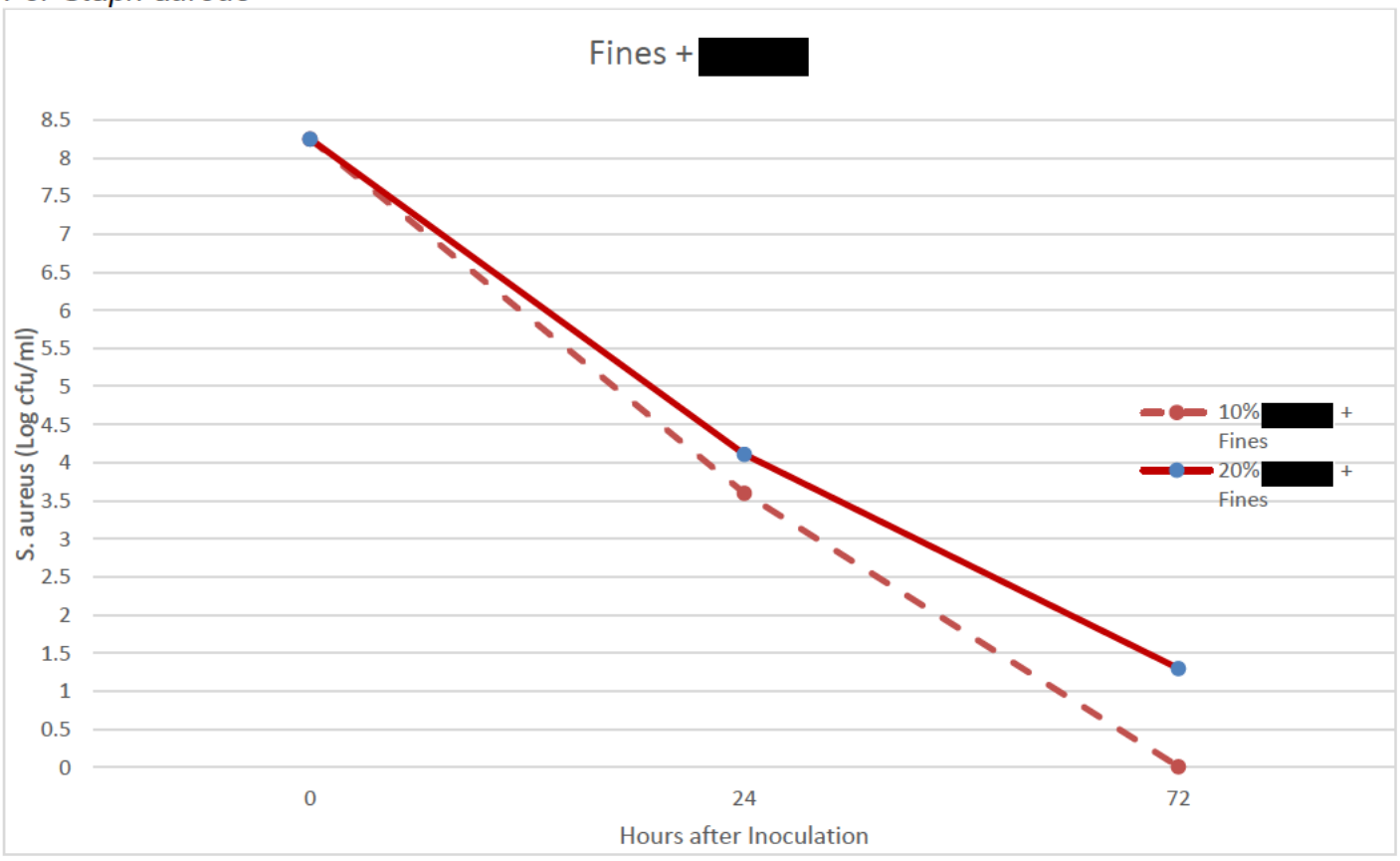


RESULTS for FINES + [redacted]

For *E.coli*

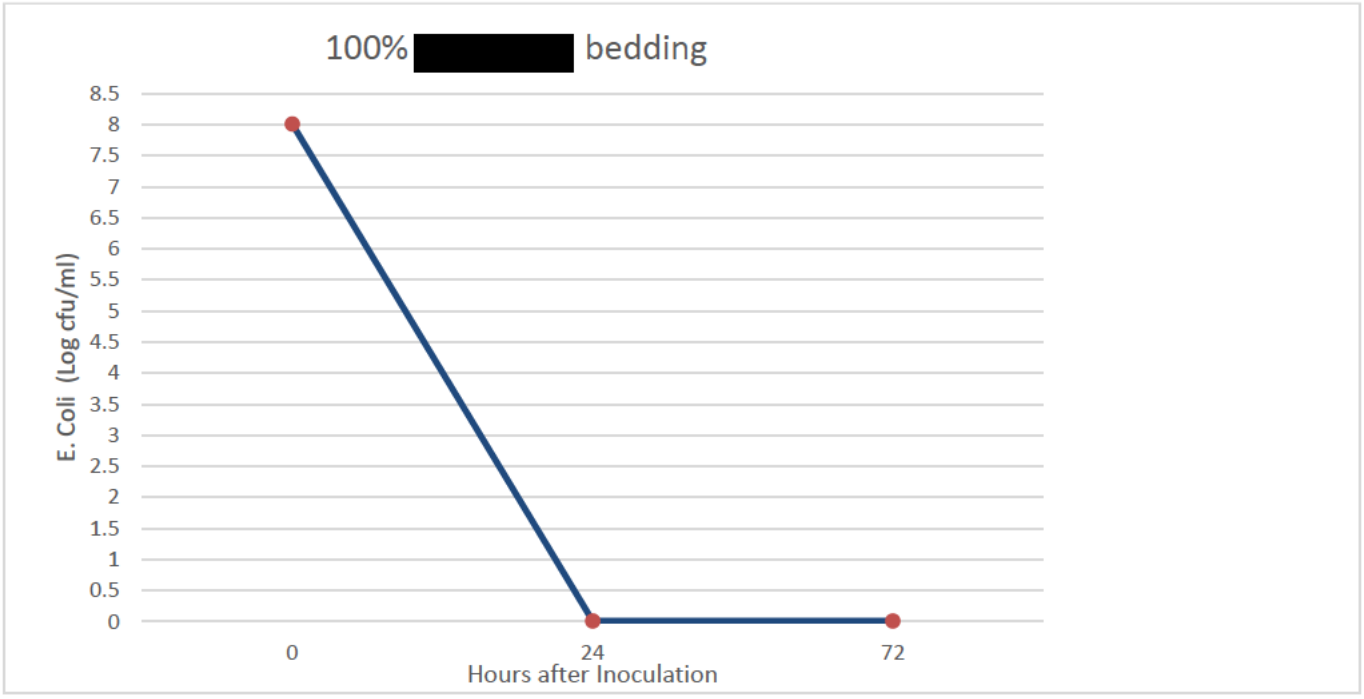


For Staph aureus

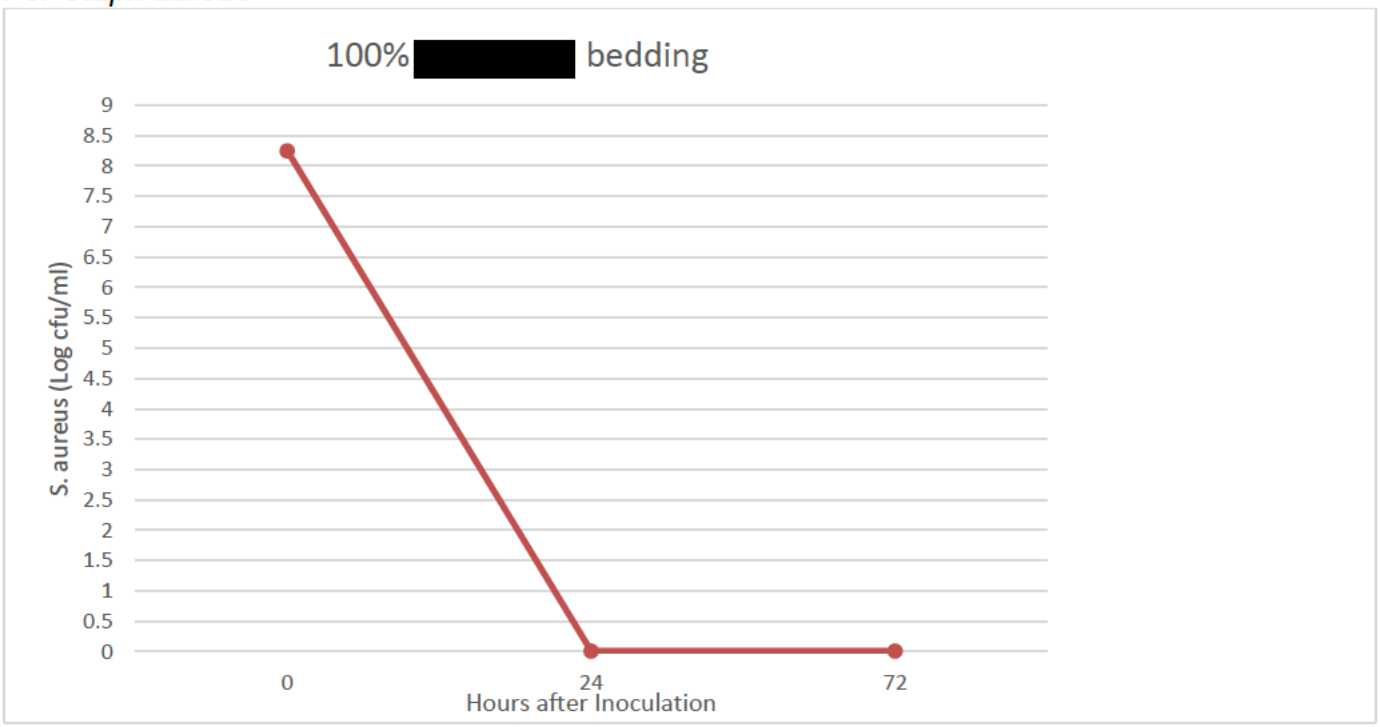


RESULTS for [redacted] BEDDING

For *E.coli*

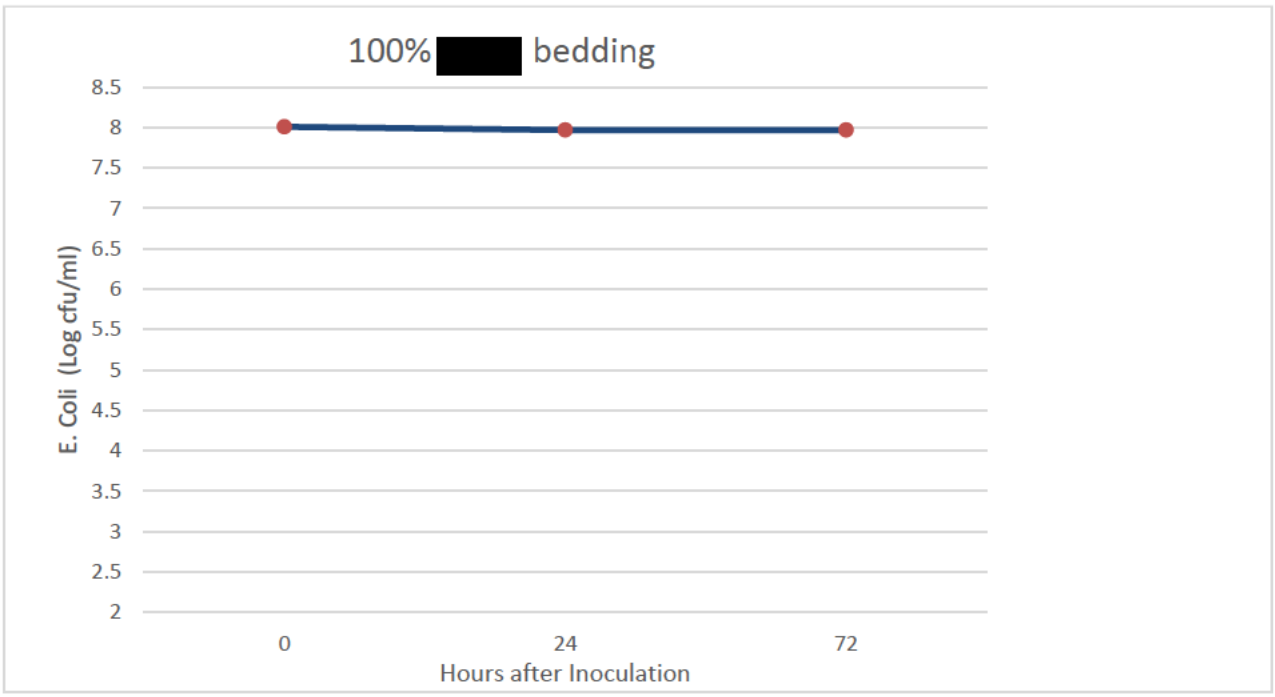


For Staph aureus

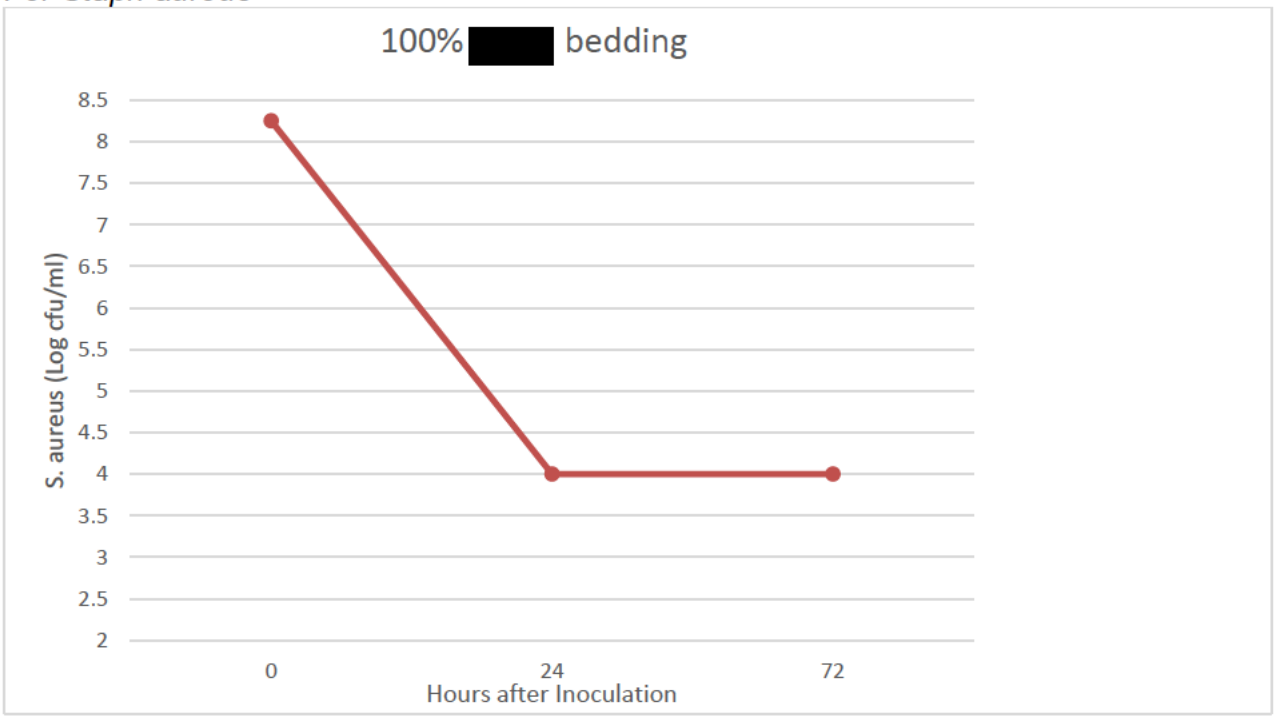


RESULTS for [redacted] BEDDING

For *E.coli*

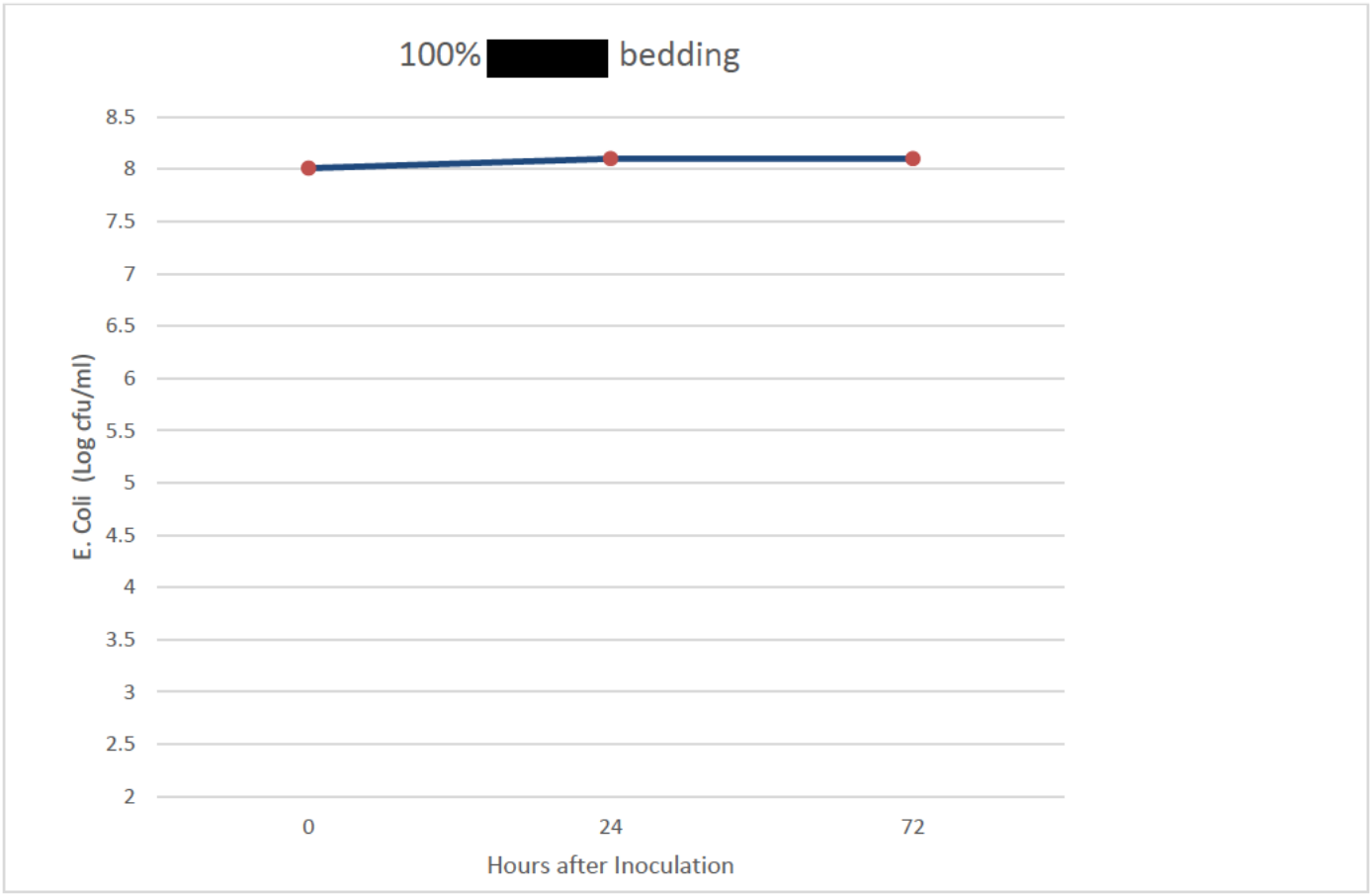


For Staph aureus

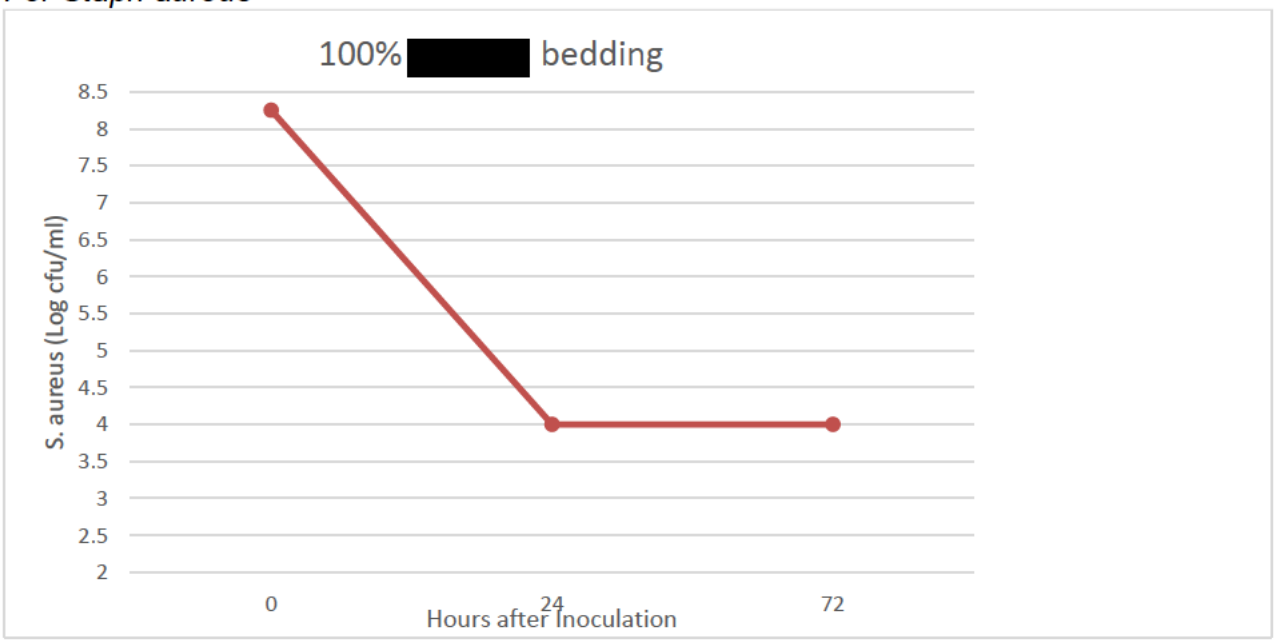


RESULTS for [redacted] BEDDING

For *E.coli*



For Staph aureus



CONCLUSIONS and DISCUSSION

To cause mastitis and other skin infections, bacteria in a cows' environment must multiply rapidly and enter a susceptible cow's teat ends or other wound. Given the opportunity, bacteria will multiply to great numbers in bedding and, if they gain access to teat ends or other leg and feet injuries, they are much more likely to be at a high enough concentration to cause mastitis and lameness. If good management keeps bacterial numbers low, and prevents bacterial access, then mastitis and lameness are prevented. Management of stalls and bedding influences the numbers of mastitis-causing bacteria in the cow's environment.

It is apparent from the results shown in the graphs above that the major factor in the reduction of the level of *E.coli* and *Staph. aureus* in bedding samples is pH. The best results were obtained from 100% Fines, or 100% Sawdust or Fines / Sawdust + [redacted] lime. This is likely entirely due to the pH extremes of pH 4 and pH 12 respectively.

Organic bedding—straw, and shavings and so forth—increase cow comfort. But they also have the potential to provide an environment that could encourage bacterial growth. There is a careful balance between maximizing cow comfort and reducing this food and water source for bacteria.

The use of sawdust as a bedding material is well documented, however, concerns over dust and water retention have led to lime based disinfection powders being added to increase the antimicrobial properties of these wood based organic materials. Researchers have shown that adding hydrated lime to sawdust or shavings increased bedding pH and reduced its water content. This hinders bacterial multiplication and daily replacement of sawdust in the back one-third of the stall also reduced bacterial numbers.

The leachate from sawdust of various tree species has been published with Oak sawdust at pH 4.2 and Pine sawdust at pH 4.5. This low pH is due to naturally occurring chemicals in the wood such as phenols, Tannins and Lignans, Resins and Terpines. Phenols are characterized by a common functional group, the phenolic hydroxyl group, this compound is water-soluble and acidic. Many of the phenolic compounds found in wood are there due to their natural micro-biocide and insecticidal properties. This might explain why the phenolic compound constitutes the most toxic compound found in wood leachate. Tannins & Lignins are substances which comprise one or more phenol functional groups. They are common in plants and have different functions; Lignin is the substance that joins the wood cells together, whereas tannins function as a defence to stop fungi and bacteria attacking the wood. Resin acids are also toxic compounds found in wood, the

different types of resin acids are also part of the trees defence system against insects and microorganisms.

From the results above it is evident that there is no benefit in adding any lime disinfectant to the Fines bedding material as its natural pH is sufficient to reduce the level of both *Staph. Aureus* and *E.coli* to undetectable from a level in 10^8 cfu/g. In fact, the addition of lime may counteract the innate biocidal properties of the raw material and add unnecessarily to the caustic skin irritation problems that these lime based products have been associated with. In addition, the use of an unadulterated, organic product may even be a marketing advantage.

Signed.....  ..... Date..... 30/09/19

Note: All work is certified under ISO9001:2008