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FINAL REPORT

Project leader: R.M. Friendship

Project Title: Tetracycline residue in pig bones causing yellowing

Objectives of Research Proposal:

To determine whether the yellow colour of bones is a predictor of the level of tetracycline present in bone or other tissues.

To determine the risk factors associated with the yellowing of bones.

Executive summary:

A trial was conducted where tetracyclines were given to 30 pigs, in 5 groups, at various dosages, for various lengths of time and via different routes of administration. Group 1 - chlortetracycline in the feed at 660 ppm for 12 weeks. Group 2 - chlortetracycline in the feed at 660 ppm for 3 weeks. Group 3 - chlortetracycline in the feed at 110 ppm for 12 weeks. Group 4 - chlortetracycline in the feed at 110 ppm for 3 weeks. Group 5 - 1 g tetracycline per 8 L water for 5 days. Group 6 - 300 mg per 45 kg body weight of oxytetracycline for 3 days. At processing the carcasses were assessed visually for bone discoloration and for UV fluorescence. Bone samples were collected at slaughter and cleaned using carrion beetles. Bones were ground to a powder and tetracycline levels measured by high-pressure liquid chromatography (HPLC). In addition bones from pigs identified at slaughter with discoloured bones were assessed for the presence of tetracyclines.

In 2006, one large packer continued to report a high number of pigs with discoloured bones (over 7000 pigs), and these carcasses required special processing. A case-control study was performed to investigate if tetracycline exposure was higher for groups of pigs being identified at slaughter with discoloured bones compared to groups of pigs from similar backgrounds delivered to the same packer but not having yellow bones.

The results of the feeding trial revealed that the HPLC test was able to distinguish between the 3 types of tetracyclines used in the trial. The levels of chlortetracycline that were measured from the bones of pigs receiving 660 ppm of chlortetracycline for 3 weeks in feed during the early grower stage ranged from 12 to 23 ppm. Levels found in bones from pigs detected at slaughter with discoloured bones were similar, averaging 23 ppm of
chlortetracycline. Pigs injected IM with 300 mg per 45 kg body weight of oxytetracycline for 3 days were found to have oxytetracycline levels of around 20 ppm. The bones from all treated animals fluoresced under ultra-violet light. This study suggests that exposure to relatively low levels for relatively short periods of time resulted in tetracycline levels present in bone at slaughter that were similar to the levels found in pigs identified with bone discolouration. Levels in liver, kidney and muscle in all treated animals were below detectable limits.

Twenty-five case herds were identified and matched with 25 herds shipping pigs to the same abattoir. Case herds were chosen on the basis of having one or more pigs identified with discoloured bones at slaughter. All case herds had a history of feeding chlortetracycline at levels of at least 660 ppm for at least 3 weeks, but many control herds had a history of tetracyclines exposure comparable to the case farms. No risk factors were identified to explain the reason some herds were found with discoloured bones and others feeding similar medication were not detected.

**Literature Review**

Tetracyclines were introduced in 1948 as broad-spectrum antibiotics that may be used in the treatment of many common infections. Tetracyclines have been widely used for growth promotion and therapeutics in animal husbandry throughout the world for over 50 years (Prescott et al, 2000). One of the side effects of tetracyclines is incorporation into tissues that are calcifying at the time of administration. They have the ability to chelate calcium ions and to be incorporated into teeth, cartilage and bone, resulting in discoloration of both the primary and permanent dentitions. This permanent discoloration varies from yellow or gray to brown depending on the dose or the type of the drug received in relation to body weight. The first report that tetracyclines persist in bones was published in 1957 (Milch et al, 1957) with other researchers describing the specific fluorescence of bound tetracyclines shortly afterwards (Buyske et al, 1960). Screening bones at slaughter using fluorescence has been advocated as a means of establishing whether pigs have been treated with tetracycline or not and to some extent the level of exposure (Samake and Matineau-Doize, 1992). In a study (Kuhne et al, 2000) examining the prevalence of fluorescence of bones in market hogs in Germany, researchers found 70% of animals tested positive. The concentration of tetracyclines ranged from 0.14 to 50.0 mg per kg from bones that were positive for fluorescence. Skeletal muscle levels of tetracyclines consistently measure below the minimum residue levels of 400 ppb and 1000 ppb for oxytetracycline and chlortetracycline when a withdrawal time of 7 days is followed. It has been suggested that meat obtained from mechanically deboned carcasses may contain bone splinters and thus be contaminated with tetracyclines. Likewise bone meal from pigs fed high levels of tetracyclines used for the preparation of animal feeds could be a concern. A complete destruction of tetracycline and chlortetracycline could not be demonstrated after heat treatment at 133 C for up to 45 min (Kuhne et al 2001). Despite the widespread use of tetracycline over a long period of time, there is very little information to suggest that the yellowness of the bone as determined by visual inspection under normal lighting conditions is related to tetracycline levels in the meat or in the
bones. In addition there is limited information regarding whether or not tetracycline residue in bone is a public health risk.

**Objectives**

To determine whether the yellow colour of bones is a predictor of the level of tetracycline present in bone or other tissues.
To determine the risk factors associated with the yellowing of bones.
To adapt and standardize an existing accredited method to do tetracycline levels in bone.
To determine whether the tetracycline breaks down after rendering.

**Materials and Methods**

1. **Controlled Feeding Trial**

Tetracyclines were administered to pigs for various lengths of time and at various dosages and using different routes of administration. The carcass was assessed for yellowing of bones and tetracycline residues in muscle, liver and bone.

*Group 1* (high exposure and long duration)
A pen of 5 pigs was fed a ration containing chlortetracycline at 660 ppm for 12 weeks from 8 weeks of age until 20 weeks of age.

*Group 2* (high exposure and short duration)
A pen of 5 pigs was fed 660 ppm of chlortetracycline for 3 weeks from 8 weeks of age until 11 weeks of age.

*Group 3* (low exposure and long duration)
A pen of pigs was fed aration containing 110 ppm of chlortetracycline for 12 weeks from 8 weeks of age until 20 weeks of age.

*Group 4* (low exposure and short duration)
A pen of 5 pigs was fed a ration containing 110 ppm of chlortetracycline for 3 weeks from 8 weeks of age until 11 weeks of age.

*Group 5* (water medication)
A pen of 5 pigs was treated with 1 gm tetracycline per 8 L of water for 5 days during the grower-finisher stage.

*Group 6* (injectable)
Five pigs were injected IM with 300 mg per 45kg body weight of oxytetracycline for 3 days during the grower-finisher stage.

Pigs from these trials were slaughtered at 24 weeks of age at the University of Guelph abattoir and the right metatarsal bone, sacral vertebrae and a rib were removed and assessed for colour. The metatarsals were ground and assessed using high-pressure liquid chromatography (HPLC) for tetracycline residues. A sample of skeletal muscle and liver will also be analysed for levels of tetracyclines.
Bones from 5 pigs identified as having discoloured bones were collected from a packing plant as well as bones from a pig with no tetracycline exposure, and these samples were also subjected to HPLC analysis.

2. Case-Control Trial

A list of shipments of pigs to a large commercial abattoir was obtained. Lots containing at least one pig identified with discoloured bones were used to select case herds. Shipments without yellow bones were used to choose control farms. Fifty farms with thorough drug use records were finally investigated. Total tetracycline per animal was calculated on each farm. Potential risk factors such as herd size, feeding system, and use of phytase was examined.

Results

Tetracyclines were not present at detectable levels in liver, kidney or muscle tissue. All bones from pigs fed tetracyclines showed some degree of fluorescence under ultra-violet light. Bones from the negative control pig did not fluoresce. Bones from the two long duration exposure experiments (high and low for 12 weeks) were not analysed because of an accident during processing. Discolouration of bone was only detected in the samples from pigs receiving 660 ppm of chlortetracycline for 12 weeks in the feeding trials. The results of HPLC analysis are presented in Table 1 and depicted in Figure 1. The levels of chlortetracycline that were measured from the bones of pigs receiving 660 ppm of chlortetracycline for 3 weeks in feed during the early grower stage ranged from 12 to 23 ppm. Levels found in bones from pigs detected at slaughter with discoloured bones were on average slightly higher, averaging 23 ppm of chlortetracycline. Pigs injected IM with 300 mg per 45 kg body weight of oxytetracycline for 3 days were found to have oxytetracycline levels of around 20 ppm.

Preparing samples to be analysed was slow and laborious. Only small bones could be used, meat was removed by hand preparation and then using carrion beetles and finally the bones were ground in a specialized mill. In all likelihood the process used to extract the tetracycline from the bone matrix was incomplete and these results are an underestimate.

In the case-control study, it was noted that all case herds had used tetracycline and at levels of 660 ppm of chlortetracycline in feed for at least 3 weeks in the grower stage. However there were control farms that used similar levels. No risk factors were identified for the discoloured bones other than moderate to high levels of tetracycline having been fed.

Table 1. Average levels of tetracyclines (ppm) in bone from pigs in various exposure groups as measured by high-pressure liquid chromatography
<table>
<thead>
<tr>
<th>Source</th>
<th>Feed 110ppm/3wks</th>
<th>Feed 660ppm/3wks</th>
<th>Water 1g/8L</th>
<th>Injectable 300 mg per 45 kg</th>
<th>unknown Discoloured bones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracycline</td>
<td>0.502</td>
<td>1.45</td>
<td>2.54</td>
<td>0.278</td>
<td>3.2</td>
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<td>Chlortetracycline</td>
<td>2.756</td>
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<td></td>
<td>22.54</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>21.4</td>
</tr>
</tbody>
</table>

**Figure 1** Average levels of tetracyclines as detected by high-pressure liquid chromatography (Low-Short = 110ppm of chlortetracycline in feed for 3 weeks, High, Short = 660ppm of chlortetracycline in feed for 3 weeks, Water = 1g of tetracycline/8L of water for 5 days, Injectable = 300 mg per 45 kg body weight, and Cases are bones taken from pig carcasses identified at slaughter with discolouration.
Discussion

The results of the feeding trial revealed that the HPLC test was able to distinguish between the 3 types of tetracyclines used in the trial, although low levels of tetracycline were recorded for pigs receiving only chlortetracycline or oxytetracycline, suggesting some interaction. There were no tetracyclines measured in the negative control bones suggesting that false positive readings are not likely a concern. However it is widely known that tetracyclines are tightly bound to the bone matrix and the extraction process may be incomplete. It is possible that this testing procedure may under-estimate the true tetracycline level. Feeding trials using radioactive labelling may be necessary to determine how significant this potential source of error might be. This technique may also be necessary to study the effectiveness of the rendering process in destroying tetracycline. We sought funding but were unsuccessful in obtaining additional support to pursue this issue.

The main tetracycline found in the discoloured bones was chlortetracycline, and the levels in these bones were on average similar to the highest levels found from the pigs on the controlled feeding trial when fed chlortetracycline at 660 ppm for 3 weeks. This suggests that the pigs with discoloured bones may not have received unusually high levels or have had long continuous exposure. The levels of tetracyclines found in the bones from the abattoir are not indicative of a problem of excessive exposure when compared to the results of the controlled study. Therefore, either the testing procedure is greatly under-estimating tetracycline levels or the discolouration is not necessarily reflective of abnormally high tetracycline exposure.

The results of the farm investigation confirmed these conclusions. Case farms included farms where chlortetracycline had been fed for only about 3 weeks at a level of 660 ppm. There did not appear to be other factors that were contributing to farms being positive for discoloured bones. Exposing pigs to relatively moderate levels or feeding for more than a short period of time can result in discolouration. The pigs are identified at the slaughter plant based on visual inspection that is subjective. This may be the main reason why lot of pigs has positive pigs and the next lot has no problem even though their exposure is identical.

Take-Home Message
Care should be taken when using tetracyclines to avoid using high levels or using them for more than a short period of time. At present this is considered a problem of cosmetics and not a public health concern and this work supports that view.

References


