

Technical Summary: The occurrence of yellow bones in swine fed tetracyclines

Chlortetracycline is a widely used feed additive in Canadian pork production. With recent disease challenges there have been increased doses prescribed by veterinarians in attempt to defeat the secondary infections in PRRS and PCV 2 virus infected herds. Other tetracyclines such as Oxytetracycline and Tetracycline are also used in swine production. Tetracyclines can cause discoloration and fluorescence when deposited in bones. As illustrated below in Figure 1, the fluorescence of the bones under UV light is quite characteristic. The incidence of yellow bones due to the tetracyclines is not related to which tetracycline or route of administration is used.

Tetracycline residues in bone are not microbiologically active [1, 2]. The tetracyclines deposited in bones are not released by typical roasting or boiling [3, 1] conditions. When tetracyclines are found in the meat levels are decreased by cooking conditions [1, 2]. These reports suggest that yellow bone is more of an appearance issue than anything else.

Recently, the Canadian Food Inspection Agency has produced two “Meat Hygiene Directives” numbers 2006-1 and 2006-12 regarding the effect of the discoloration on meat quality. See: <http://www.inspection.gc.ca/english/anima/meavia/mmopmmhv/direct/2006/direct12e.shtml> . The agency was convinced by evidence presented by Ontario Pork that the information in the literature was valid and changed its opinion to conclude that yellow bone is a quality defect.

Figure 1: Bone fluorescence due to tetracyclines.



Picture courtesy of OMAFRA

The tetracyclines deposit in bone since they are excellent chelating agents [4]. Chelates arise when metals are able to form a ring type structure with an organic molecule in solution. These structures can be very stable. In the case of the tetracyclines, stable chelates are formed with the group IIa metals (magnesium, calcium, strontium, etc) which also fluoresce. Fluorescence is the absorption of light at one wavelength which is then emitted at another lower energy wavelength. The fluorescence of metal chelates of the tetracyclines is also a very well known property of the tetracyclines and forms the basis for a number of analytical methods [5]. There are variations in fluorescence intensities due to molecular structure and pH [6] however the three tetracyclines commonly used in swine production appear to exhibit very similar intensities of fluorescence at physiological pH.



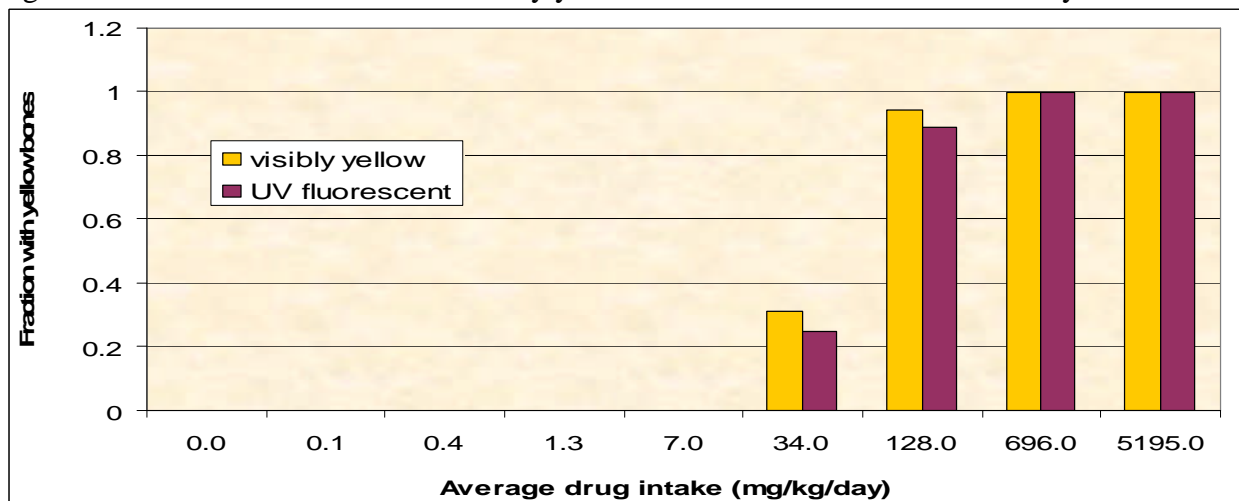
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The tetracyclines have been known to form a fluorescent complex in bones since the late 1950s [7]. Tetracyclines deposit in areas of bone formation and are part of the mineralized bone. Physiologists have used timed injections of tetracyclines to delineate growth patterns in bones by fluorescence microscopy ever since [8].

German researchers have used UV fluorescence of bone surfaces to detect animals treated with tetracyclines for decades [9]. A survey in 2000 showed that of 17150 “fattening pigs” examined 30% showed no visible fluorescence, while 29.5% showed fluorescence from 20% or less of the vertebrae and ribs, 25.1% showed fluorescence from 20-80% of the vertebrae and ribs and 15.4% showed fluorescence from more than 80% of the vertebrae and ribs. Unfortunately the extent of the fluorescence was not related back to bone levels or treatment regimens. The only study that related bone concentration of Chlortetracycline at slaughter to feed levels in swine was conducted using low levels of drug [3].

A 2 year toxicity study of rats fed varying doses of Chlortetracycline published in the early 1960s found that the occurrence of visibly yellow or fluorescent bone was dependant on dietary Chlortetracycline intake [10]. The study examined a broad range of physiological and toxicological parameters at various levels of CTC in the diet ranging from 0.0001% to 5.0% by weight (1 ppm to 50,000 ppm). The results for fluorescence and visible yellowing of the bone were similar: the effect is not observed until a certain dietary threshold is reached. In both male and female rats there is no visible yellowing or fluorescence of the bone observed below the level of 7 mg/kg/day of Chlortetracycline in animals that survived the full two years. Figure 2 illustrates this trend for male rats that were sacrificed at the end of the study.

Figure 2: Fraction of male rats with visibly yellow or UV fluorescent bones after 2 years



Data from Dessau and Sullivan 1961

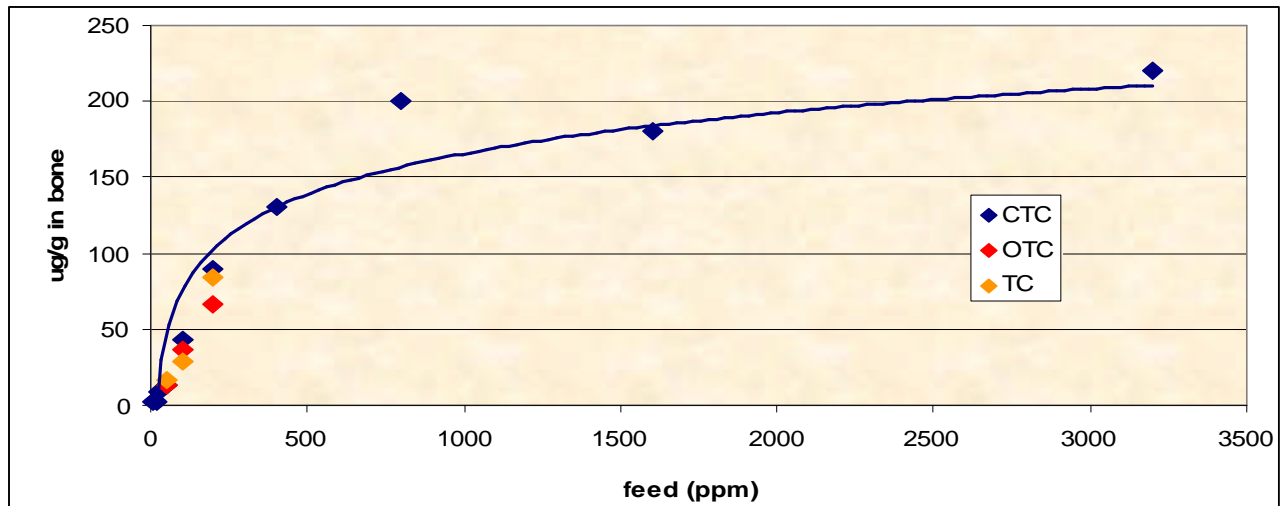
Below 34 mg/kg/day Chlortetracycline was deposited in the bone but the bone had to be ground up and extracted to detect the Chlortetracycline fluorescence. Visibly yellow or fluorescing bones therefore are dependant on the dosage.

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This concentration dependence is well known from the physiological literature where the doses required to label bone are documented. Very low doses of Tetracycline (1.2 and 4.8 mg/kg/day) over 90 days do not result in detectable fluorescent labelling of the bone [11]. In adult swine, intravenous infusions of 25 mg/kg bodyweight of Oxytetracycline were used to label the femur of adult swine with a detectable fluorescent layer [12]. Injections of 20 mg/kg bodyweight of Tetracycline were used with 11 week old pigs [13].

Studies in chickens confirmed that the bone concentration was dependant on the feed intake [9]. These results are illustrated in figure 3. The chicken data suggest that there will be a maximum concentration of Chlortetracycline in bone.

Figure 3: Tetracyclines in 6 week old chicken bones by feed concentration



Data from Bruggemann et al 1966

In turkeys there is a rough correlation between fluorescence intensity and bone concentration of tetracyclines however the ranges are broad and overlapping [9b].

Recently, the UK's Central Science laboratory has published a paper demonstrating that the effects of long term dosing can be differentiated from single therapeutic treatments by the pattern of the fluorescence in the bones [14]. They have demonstrated that long term administration of Oxytetracycline in swine results in a wide, diffuse band of fluorescence. Short term therapeutic administration at 10 times the prophylactic concentration resulted in sharp fluorescent bands. Results in chickens showed that both Oxytetracycline and Chlortetracycline behaved similarly.

Table 1: Approximate Doses of Chlortetracycline from Feed.

Body weight ¹ (kg)	Feed ² (kg/day)	Approximate daily CTC intake (mg/kg bw/day) per dose (ppm)							
		110	220	330	440	550	660	1100	1210
30	1.54	5.6	11.3	16.9	22.6	28.2	33.8	56.4	62.1
42	1.78	4.7	9.3	14.0	18.6	23.3	27.9	46.6	51.2
60	2.23	4.1	8.2	12.3	16.3	20.4	24.5	40.8	44.9
80	2.55	3.5	7.0	10.5	14.0	17.5	21.0	35.0	38.5
100	2.67	2.9	5.9	8.8	11.7	14.7	17.6	29.3	32.3

1) Body weight as midpoint of range

2) F/C =2.6

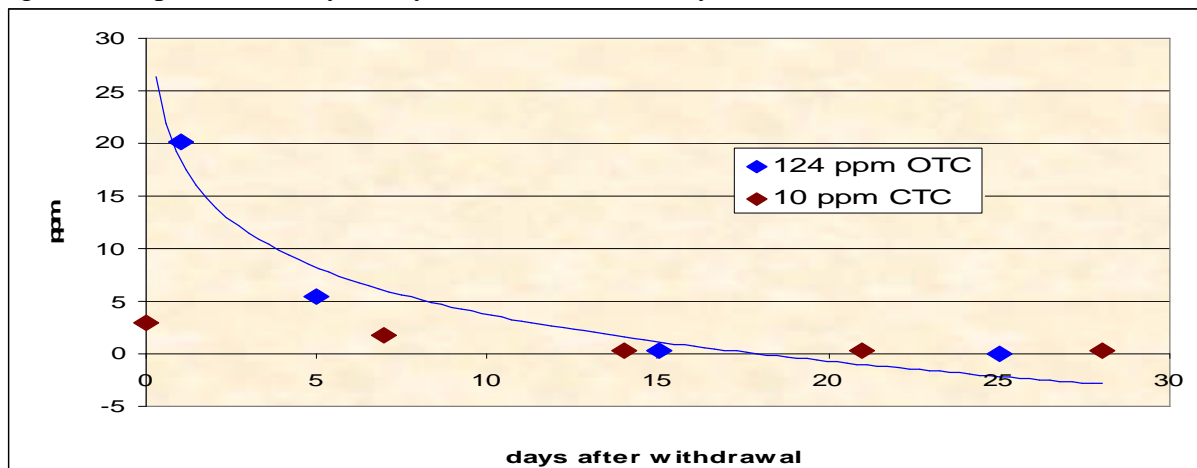
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Table 1 shows the approximate daily doses of Chlortetracycline that are available based on a common feeding mixed sex feeding program. There are currently no data sets such as that shown in Figure 3 for swine. Based on the results of the rat studies it would appear that animals fed doses above 10 mg/kg bw/day are at risk of yellow bone and those in the higher dosage ranges should be expected to exhibit at least some visible yellowing and fluorescence of the bone. A dose of 10 mg/lb bodyweight per day of Chlortetracycline has been approved for use in feed in the United States however, a review of the Freedom of Information summary [16] indicates that no human safety information was submitted or required and the clinical endpoints were assessed based on clinical observations not necropsy so there is no indication if yellowing or fluorescence of bones could be expected at this level or not.

Bone is not a static tissue. Resorption is the process of breakdown of bone mineral to release calcium for metabolic use or the formation of new bone. Studies with tritium labelled tetracycline in rats have shown that the labelled tetracycline is depleted from the soft tissues in about 24 hours [15]. The depletion of radioactivity from the bone follows a pattern that indicates at least two processes are at work: a “fast” exchange process and a “slow” process due to bone resorption. In young rats this process took about 70 days to deplete the labelled tetracycline.

Similarly, skeletal levels of tetracyclines in chickens decline after the end of administration as shown in Figure 4 [9b, 3]. It is important to note that turnover of bone in birds is thought to be much higher in birds than mammals due to structural differences [17].

Figure 4: Depletion of Oxytetracycline and Chlortetracycline from Chicken bones



CTC data: Bruggemann et al 1966; OTC Kuhne et al 2000

So, an extended withdrawal period should produce some depletion of tetracyclines from the skeleton of swine but will not completely remove all fluorescence. The other effect of withdrawal, as seen in the photomicrographs in the paper from the UK Central Science Lab [14], will be to allow new layers of bone to form. Anecdotal evidence from producers suggests that cutting back to lower levels of Chlortetracycline for the final phase of finishing may decrease the risk of the outer layers of bone being visibly yellow. Ontario Pork is undertaking studies to determine some of the contributing factors for yellow bone in more detail.



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