Induction of Tibial Dyschondroplasia in Turkeys by Tetramethylthiuram Disulfide (Thiram)

S. Simsa,* A. Hasdai,+ H. Dan,* and E. M. Ornan*†

*Faculty of Agricultural, Food and Environmental Quality Sciences, Department of Biochemistry and Nutrition, The Hebrew University, Rehovot 76100, Israel; and †Institute of Animal Science, The Volcani Center, Bet Dagan 50250, Israel

ABSTRACT Tibial dyschondroplasia (TD) is a prevalent skeletal abnormality associated with rapid growth rate in many avian species; it causes enormous economic losses and is an animal welfare problem. Tibial dyschondroplasia is characterized by the presence of a nonvascularized, nonmineralized lesion that extends from the epiphyseal growth plate into the metaphysis of the proximal tibiotarsal bones. The mechanisms underlying TD development are not known, although they have been extensively studied in broilers using different induction models. However, an effective model for TD induction in turkeys has never been described. The objective of this study was to establish such a model by using tetramethylthiuram disulfide (thiram), an agent that is frequently used in broilers to induce TD. We found that dramatically longer exposures to much higher concentrations of thiram were required to induce TD in turkeys vs. broilers. In contrast to broilers, in which 50 mg/kg of thiram induces a high incidence of severe TD within 10 d, in turkeys, an exposure to 400 mg/kg of thiram for 11 wk was necessary for the development of severe TD lesions. These results show different mechanisms for TD induction in these 2 closely related species, suggesting differences in TD etiology between them.

Key words: tibial dyschondroplasia, thiram, growth plate

INTRODUCTION

Tibial dyschondroplasia (TD) is a prevalent skeletal abnormality associated with rapid growth rate in many avian species, especially broilers (Leach and Lilburn, 1992) and turkeys (Wyers et al., 1991); it causes enormous economic losses and is an animal welfare problem (Pines et al., 2005). The disorder, first described by Leach and Nesheim (1965), is characterized by the presence of a nonvascularized, nonmineralized lesion that extends from the epiphyseal growth plate into the metaphysis of the proximal tibiotarsal bones (Leach and Nesheim, 1965, 1972). Poulos (1978) was the first to describe TD in turkeys: he characterized a disorder that was morphologically similar to that of dyschondroplasia in broilers and concluded that it was TD.

Tibial dyschondroplasia is a leading cause of lameness in meat-type poultry due to growth plate fracture, infection, and bone deformation (Lynch et al., 1992). However, Hocking et al. (2002a) showed a high incidence of TD in turkeys with no gait abnormalities, making the precise relevance of high-TD incidence to turkey welfare unclear. The mechanisms underlying TD development are not known, although several theories have been advanced to explain its etiology. Rath et al. (2005) suggested that a metabolic dysfunction leads to destruction of blood capillaries in the transition zone. Praul et al. (2000) suggested that the lesion occurs when the transition of chondrocytes from prehypertrophy to hypertrophy is inhibited; similarly, Webster et al. (2003) suggested that the lesion is filled with transitional chondrocytes that fail to differentiate. Orth and Cook (1994) suggested that the chondrocytes secrete an immature cartilage that becomes highly cross-linked and is resistant to resorption and vascularization by the metaphyseal vessels. We recently showed that the defective chondrocytes fail to synthesize and secrete enzymes from the matrix metalloproteinase family, and thus, the matrix is not properly degraded, fewer blood vessels penetrate into the growth plate, calcification is inhibited, and the nonvascularized, nonmineralized TD lesion is formed (Simsa et al., 2007a,b).

There are various tools for the study of TD. In broilers, many studies use TD induction models such as low dietary Ca:P ratio (Rennie et al., 1993), Cys supplementation (Bai and Cook, 1994), Cu-deficient diets (Orth et al., 1994), and the use of dithiocarbamates such as thiram (Rath et al., 2004). The latter is one of the most commonly used agents for TD induction in broilers. Since 1986, thiram has been used for rapid and effective TD induction. Veltman and Linton (1986) showed that TD inci-
Figure 1. Effect of thiram on tibial dyschondroplasia (TD) incidence in broilers and turkeys. Panels A and B: 39 broilers were raised on either a normal diet (control) or one containing 50 mg/kg of thiram for 10 d. At the end of this period, all tibial growth plates were dissected and scored for TD severity. Panel A: relative percentages of each score. Panel B: average score for each group. The 2 groups were statistically different. Panels C, D, E, and F: 30 turkeys were raised on a normal diet (control), a diet containing 100 mg/kg of thiram, or one containing 400 mg/kg of thiram for 7 or 11 wk. After 7 and 11 wk, the tibial growth plates of 15 birds from each group were dissected and scored for TD severity. Panels C and E: relative percentages of each score. Panels D and F: average score for each group. The control and 100-mg/kg groups were not statistically different, whereas the 400-mg/kg group was statistically different at all time points.a,b Means with different letters are significantly different (P < 0.05).

dence is significantly increased in chicks fed with either 30 or 60 mg/kg of thiram at as early as 2 wk of age. Orth et al. (1994) showed a substantial increase in TD induction in broilers fed with 25 mg/kg of thiram for 3 wk. Rath et al. (2005) showed that 95% of chicks fed 100 mg/kg of thiram for 2 d, when they were 7 d old, developed severe TD lesions at the age of 16 d.

No such effective model has been described for TD induction in turkeys (Hocking et al., 2002b); therefore, most studies are based on field TD (Julian, 1985; Wyers et al., 1991; Rath et al., 1994; Knopov et al., 1997). However, samples from field trials are taken at the final stage of the disease, when lameness is obvious and the lesions are large. At this stage, it is impossible to distinguish between the primary and secondary events leading to lesion formation.

The objective of this study was to establish a research model for the investigation of TD in turkeys using thiram as the inducing agent.

MATERIALS AND METHODS

Birds and Diets

One-day-old Cobb-strain broiler chicks (n = 39) were obtained from a commercial hatchery (Brown Hatcheries, Hod Hasharon, Israel), and they were raised for 10 d under the recommended temperature regimen and fed diets according to NRC recommendations ad libitum. During this period, the birds were fed either of the following: 1) a regular diet (control group) or 2) the same diet containing 50 mg/kg of tetramethylthiuram disulfide (thiram; Sigma Chemical, St. Louis, MO).

One-day-old BUT-strain turkey chicks (n = 30) were obtained from a commercial hatchery (Ramit, Hadera, Israel), raised for 7 or 11 wk under the recommended temperature regimen, and fed according to NRC recommendations ad libitum either of the following: 1) a regular diet (control group), 2) the same diet containing 100 mg/kg of thiram, or 3) the same diet containing 400 mg/kg of thiram.

Evaluation of TD

After the indicated times (10 d for broilers, 7 or 11 wk for turkeys), birds were slaughtered, and the proximal growth plates of the tibia were shaved longitudinally to determine the incidence and severity of TD. The severity was scored subjectively as follows: 0 = healthy growth plate; 1 = recognizable cartilage plaque; 2 = plaque covering up to 20% of the longitudinal section; 3 = plaque covering up to 50% of the longitudinal section; and 4 = plaque covering up to 80% of the longitudinal section. In addition, the average score was calculated for each group by summing the total score for all the legs in the group and dividing it by the number of legs. Representative growth plates for each score were photographed. All procedures were approved by the Animal Care Welfare Committee.

Evaluation of Lameness

Each week, all pens were watched for lame birds. Sitting birds were forced to rise, and their walking abili-
ties, as well as the period they were able to remain standing, were tested.

**Feed Intake and BW**

Total feed intake was measured for each group at 10 d for broilers or at 7 and 11 wk for turkeys and divided by the number of birds to obtain individual food intake. Individual BW were recorded at those time points, and mean pen food intake was determined at those time points.

**Statistical Analysis**

Differences in TD scores and average scores were analyzed by $X^2$ test. The effect of thiram on BW was analyzed by t-test in broilers or ANOVA in turkeys. All statistical analyses were carried out with JMP software (SAS Institute, 2000).

**RESULTS**

**Thiram Induces TD Differentially in Broiler and Turkey Growth Plates**

We used thiram to induce TD in broilers and turkeys. In the broilers, after 10 d of thiram administration, 56% of the tibias showed severe TD lesions (scores 3 and 4), 34% showed light TD lesions (scores 1 and 2), and the rest of the group (10%) had no TD lesions. The average score in this group was 2.53 (Figure 1, panels A and B). In the control group, all the tibias had a normal phenotype; thus, the average score was 0 (Figure 1, panels A and B). Repeating this protocol in turkeys did not induce TD (data not shown); therefore, we increased the thiram dosage to 100 or 400 mg/kg and the period to 7 and 11 wk. Up until wk 7, no TD was observed. At the age of 7 wk, 70% of the tibias in the 400-mg/kg group showed light TD lesions (score 1). The average score was 0.7 (Figure 1, panels C and D). The 2 other groups (control and 100 mg/kg of thiram) had a normal phenotype, and the average score was 0 (Figure 1, panels C and D). At the age of 11 wk, 40% of the tibias in the control group showed spontaneous TD of light severity (scores 1 and 2), with an average score of 0.53 (Figure 1, panels E and F). In the 100-mg/kg group, 30% of the tibias had light-severity TD lesions (score 1), with an average score of 0.3 (Figure 1, panels E and F). These 2 groups were not statistically different. In the 400-mg/kg group, 54% of the tibias had severe TD lesions (scores 3 and 4), 30% had light TD lesions (scores 1 and 2), and the rest (16%) had normal growth plate phenotypes. The average score was 2.53 (Figure 1, panels E and F). This group was statistically different from the others. Representative growth plates of each group are shown in Figure 2.

**Turkeys with Thiram-Induced TD are Not Lame**

Because lameness in poultry is strongly associated with TD lesions (Lynch et al., 1992), the number of lame birds was counted in each group. In the broilers, there was a positive correlation between TD and lameness;
Thiram reduces food intake and BW

Food intake was lower in the thiram-fed groups in both broilers and turkeys. For broilers, the average food intake was 148.5 g in the control group and 100.6 g in the 50-mg/kg of thiram group (Figure 3, panel A). For turkeys, at 7 wk, the average food intake was 305.2 g for the control group, 271.2 g for the 100-mg/kg of thiram group, and 196.2 g for the 400-mg/kg of thiram group (Figure 3, panel B). At 11 wk, the average food intake was 433 g for the control group, 377.1 g for the 100-mg/kg of thiram group, and 356 g for the 400-mg/kg of thiram group (Figure 3, panel C). Accordingly, BW (±SE) were also lower in the thiram-fed birds. For broilers, the average BW at 10 d was 241.1 ± 1.97 g for the control group and 180.3 ± 3.42 g for the 50-mg/kg of thiram group. Those 2 groups were significantly different (Figure 4, panel A). For turkeys, at the age of 7 wk, the average BW for the control group was 3,295 ± 158.12 g, the average BW for the 100-mg/kg of thiram group was 3,098.846 ± 134.4 g, and the average BW for the 400-mg/kg of thiram group was 2,502.308 ± 279.67 g. The control and 100-mg/kg groups were not significantly different; the 400-mg/kg group was significantly different (Figure 4, panel B). At 11 wk, the average BW for the control group was 6,821.53 ± 279.3 g, the average BW for the 100-mg/kg of thiram group was 6,076.92 ± 292.64 g, and the average BW for the 400-mg/kg of thiram group was 4,687.5 ± 183.02 g. All groups were significantly different (Figure 4, panel C).

DISCUSSION

This study examined the efficiency of thiram as a TD inducer in turkeys to establish a research model for TD in this species. Previous studies have used field-detected TD to characterize the disorder in turkeys (Julian, 1985; Wyers et al., 1991; Rath et al., 1994; Knopov et al., 1997), an approach that has several disadvantages. First, the percentage of afflicted birds in each flock is unpredictable and variable, making continuous research problem-
atic. Second, only lame birds are selected for examination, and TD-infected birds that are not lame can be overlooked. Third, and most important, when the lesion is in its advanced stages (once the bird is already lame), it is impossible to distinguish between the primary and secondary events leading up to it. Because TD etiology is unclear, it is crucial to understand the key steps involved in its onset. Such questions can only be answered by finding a model for effective and rapid TD induction.

To the best of our knowledge, only 1 study has examined possible TD-inducing agents in turkeys. Hocking et al. (2002b) studied the role of Ca and available P in TD etiology in turkeys by using 16 diets containing different concentrations of these minerals; they concluded that dietary Ca and P do not affect the prevalence of TD.

Thiram is a very efficient TD-inducing agent in broilers (Veltmann and Linton, 1986; Rath et al., 2004, 2005), and we therefore studied its effectiveness for TD induction in turkeys. Longer exposure to markedly higher concentrations of thiram was required in turkeys vs. broilers for TD induction. Surprisingly, 50 mg/kg of thiram, a dosage that induced a high incidence of severe TD in broilers within 10 d, did not induce TD in turkeys. We increased the thiram dosage to 400 mg/kg, and it took a full 11 wk on this diet before the turkeys showed severe TD lesions. Furthermore, at this stage, spontaneous mild TD was also detected in the control group. Because TD incidence peaks in turkeys from 10 to 12 wk (Poulos, 1978), we conclude that in turkeys, thiram can affect the severity of TD incidence, but it cannot serve as an inducer for studies on initiation of this disorder.

Another important finding was that in contrast to the TD-affected broilers, which showed noticeable gait abnormalities and lameness, the TD-affected turkeys were not lame. These results are in agreement with Hocking et al. (2002a,b), who reported a high incidence of TD in turkeys with no gait abnormalities. It is therefore not clear whether TD is an animal welfare issue in turkeys as it is in broilers; however, TD lesions can be a primary location for the development of osteomyelitis (Wyers et al., 1991), which is the most common cause of long-bone necrosis in turkeys (Julian, 1985). So even if the TD lesions themselves are not a welfare problem for turkeys, they can serve as a platform for other pathogeneses.

Based on morphological characteristics, TD is addressed as a similar disorder in broilers and turkeys (Poulos, 1978). Recently, we found developmental differences (Simsa and Ornan, 2007) between broiler and turkey growth plates. Here we show different mechanisms for TD induction, suggesting differences in TD etiology between these 2 closely related species.

REFERENCES


