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Effects of cold stress on growth performance, carcass traits and tibia attributes in broiler chickens with thiram-induced dyschondroplasia

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Abstract

The objective of this study was to investigate the effect of cold stress (CS) on growth performance and tibia attributes in broiler chickens with thiram-induced dyschondroplasia (TD). Four hundred 10-day-old male broilers were randomly allocated into four groups including, NT0: normal temperature (NT) without thiram; NT50: NT + thiram; CS0: CS without thiram; and CS50: CS + thiram in a completely randomised. The birds in CS groups were placed at a constant temperature of 15 ± 1°C during 11–20 days. Thiram (50 mg/kg) was added to the diet during 11–14 days to induce TD. Results showed that main effects of CS and thiram significantly decreased body weight and daily weight gain during 11–42 days ($p < 0.05$). Feed intake in the thiram50 group was significantly lower than the group thiram0 during 25–42 days ($p < 0.05$). Feed conversion ratio in CS birds was significantly more than NT group during 25–42 days ($p < 0.05$). On day 16, tibia width (TW) and TW to tibia length (TL) ratio were significantly higher in CS chicks compared to the NT group. TW was significantly higher in thiram50 group than thiram0 group ($p < 0.05$). On day 19, TL in CS chicks was significantly shorter than NT ($p < 0.05$). On day 23, growth plate width (GPW) in thiram50 group was significantly higher than thiram0 birds. In general, thiram increased tibial GPW and CS decreased TD severity as well as decreased growth performance in broilers.

KEYWORDS
broilers, cold stress, performance, thiram, tibial dyschondroplasia

1 | INTRODUCTION

Thiram-induced dyschondroplasia (TD) is the most common bone development problem in fast-growing broilers. It was first reported in turkeys in 1978. This abnormality disrupts the long bones growth plates in broilers, ducks, and turkeys. Statistical surveys have shown that TD in chickens and turkeys causes approximately 30% of deaths and is responsible for the high economic losses in the poultry industry (Dan et al., 2009; Leach & Monsonego-Ornan, 2007).

Dyschondroplasia may occur in the anterior and posterior regions of the femur, posterior tibia, anterior tibia, and anterior humerus, but is less common (Barroeta et al., 2012). This disorder causes bone flexion in the anterior part of the tibia (deformed bones) and lameness. Eventually, the affected birds die due to a lack of mobility to access water and feed. If broilers are kept until they reach the desired weight, the damage caused by dyschondroplasia becomes more severe and causes paralysis (Barroeta et al., 2012). Birds with severe collisions are likely to be more susceptible to fractures during handling and ultimately lead to increased costs (Pines & Reshef, 2015). Symptoms of this disease include the appearance of a cartilaginous mass devoid of blood vessels, minerals, and opaque white in the growth plates of long bones that extends into the anterior metaphyseal region of the tibiotarsus and in some cases tarsometatarsus (Huang et al., 2019; Jiang et al., 2020; Waqas et al., 2020).

TD has been induced by different protocols. This complication can be caused by nutritional factors such as changes in dietary calcium to phosphorus ratio, dietary supplementation of cysteine, and feeds contaminated with Fusarium. Also, various dithiocarbamates such as thiram have been used successfully to induce TD. In most recent studies, TD in chickens has been induced using thiram (Iqbal et al., 2018; Jiang et al., 2020; Li et al., 2007; Mehmood et al., 2019).

Thiram is a fungicide that is typically used in farming (Jahejo et al., 2020). This chemical compound weakens the cartilages and causes lameness (Rasaputra et al., 2013), induces chondrocyte proliferation and disturbs the endochondral calcification in the growth plate of the broiler chickens (Tian et al., 2008).

According to previous researches, chondrocytes are unable to complete normal bone calcification during skeletal growth due to the absence of angiogenesis in the proximal tibial growth plates of TD chickens, and fewer dead chondrocytes from the area can be transported through blood vessels in a timely manner (Huang et al., 2019). Then, a CS may enhance vascularisation (Atay et al., 2020) in growth plates and alleviates TD. The mild CS had no adverse effects on growth performance but improved the immunity and adaptability to acute CS in broilers (Su et al., 2020). Keeping broilers for a period of time in CS conditions during the rearing period is likely reduces fuel costs to heat poultry farms (Gad et al., 2020). Fuel cost is one of the main obstacles to poultry production in cold regions of the world. In addition, with the increasing success of outdoor breeding systems, CS will be of greater interest to researchers (Campo et al., 2008; Tsiouris et al., 2015).

In recent years, CS has attracted a lot of attention in poultry farming. Considering the contrasting effects of thiram and CS on tibia length (TL) and growth plate width, we hypothesised that CS had an effective function in the tibia and prevented dyschondroplasia in broilers. Therefore, the aim of this study was to investigate the effects of CS on growth performance, carcass traits and tibia attributes **in broilers** with **thiram-induced tibial dyschondroplasia**.

2 | MATERIALS AND METHODS

2.1 | Birds, diets and housing

All procedures were approved by the Animal Care and Use Committee of the Ferdowsi University of Mashhad, Mashhad, Iran, (Ethical approval code: IR.UM.REC.1399.042).

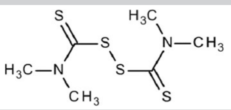
A total of 400 1-day-old male broilers from Ross 308 strain with an average weight of 45.97 ± 1.15 g were selected from a commercial hatchery. The chicks were transferred to the Livestock and Poultry Research Station of the Ferdowsi University of Mashhad, Iran, at an altitude of 990 m above sea level, placed in battery cages, and regularly reared for up to 10 days. At 11 days of age, the birds were randomly divided into four groups with a mean weight of 197.6 ± 21.5 g. Each treatment was replicated five times with 20 broiler chicks per replicate in a completely randomised design (CRD) as a 2 × 2 factorial arrangement.

The treatments included (1) Group reared at normal temperature (NT) without the addition of thiram to the diet (NT0). These birds were reared in a normal temperature condition during the whole experimental period. Temperature treatment was designed according to any preliminary experiment performed before the treatments. On day one of age, the temperature of the rearing house was set at 33°C, and it was gradually reduced by 0.5°C a day to 21°C. It was kept constant at this temperature after that. (2) NT group and adding thiram to the diet (NT50): Temperature conditions were the same as NT group. These birds received a dose of 50 mg/kg thiram in diet from 11 to 14 days of age. (3) Cold stress (CS) group without thiram in diet (CS0): birds in this group were exposed to 15 ± 1°C at the age of 11–20 days. The temperature of the rearing house before and after CS period was similar to the NT group. (4) CS group with adding thiram to the diet (CS50): Temperature conditions were the same as CS group. Birds in this group received the thiram at a dose of 50 mg/kg diet from 11 to 14 days of age (Mehmood et al., 2018). Each group was replicated five times with 20 chicks per replicate. Main effects in this experiment included NT, CS, Thiram0 and Thiram50. Tetramethylthiuram disulphide (thiram) was prepared from the Merck Company (Table 1).

The basal diets were formulated according to Ross 308 nutrient recommendations (Aviagen, 2014b; Table 2). All chickens were reared up to the age of 10 days according to the recommendation of Ross 308 strain (Aviagen, 2014a) and they were fed with a standard diet (Aviagen, 2014b). The cage size was 2 × 1 × 0.6 m (L × W × H) furnished with a paper roll as bedding material. Each cage was equipped with four nipple drinkers and two tray feeders for the first 5 days, and after that a tube feeder was placed in front of the cages. The birds were weighed on a group basis in each cage at 10 days of age, the end of the grower (24 days), and finisher (42 days) periods.

Average weight gain (WG), feed conversion ratio (FCR) and daily mortality was also calculated during the experiment. The mean WG

TABLE 1 Physical and chemical properties of thiram used in this study[a]

Name	Chemical structure	Chemical formula	Molarity	Water solubility at 20°C, mg/L	Boilig point, °C	Melting point, °C	Flash point, °C
Tetramethylthiuram disulphide		$C_6H_{12}N_2S_4$	240.44	16.5	129	148–152	89

[a]Based on the information provided by supplier company (Merk Company).

TABLE 2 Ingredients and nutrient composition of experimental diets, *as-fed basis*

Ingredients (%)	Starter (1–10 days)	Grower (11–24 days)	Finisher (25–42 days)
Corn (8.5% CP)	52.92	53.16	58.43
Soybean meal (44% CP)	40.83	37.62	32.09
Soy oil	1.74	5.19	5.72
Dicalcium phosphate	1.53	1.34	1.18
Limestone	1.44	1.20	1.09
Common salt	0.30	0.30	0.30
Sodium bicarbonate	–	0.10	0.10
Mineral premix[a]	0.25	0.25	0.25
Vitamin premix[b]	0.25	0.25	0.25
DL-methionine	0.43	0.35	0.32
L-Lysine HCl	0.23	0.22	0.23
L-Threonine	0.08	0.02	0.04
Calculated composition, (%)			
Metabolisable energy (kcal/kg)	3000	3100	3200
Crude protein	23.0	21.5	19.5
Calcium	0.96	0.87	0.79
Available P	0.48	0.43	0.395
Methionine	0.77	0.68	0.63
Methionine + Cystine	1.08	0.99	0.91
Lysine	1.44	1.29	1.16
Threonine	0.97	0.88	0.79

[a]Vitamin premix provided per kilogram of diet: vitamin A (retinyl acetate), 15,000 IU; vitamin D_3, 5000 IU; vitamin E (DL-α-tocopheryl acetate), 80 mg;vitamin K, 5 mg; thiamin, 3 mg; riboflavin, 10 mg; pyridoxine, 5 mg; vitamin B_{12}, 0.02 mg; niacin, 70 mg; choline chloride, 1800 mg; folic acid, 2 mg; biotin, 0.4 mg; pantothenic acid, 20 mg.

[b]Mineral premix provided per kilogram of diet: Mn (manganese sulphate), 100 mg; Zn (zinc sulphate), 65 mg; Cu (copper sulphate), 5 mg; Se (Sodium Selenite), 0.22 mg; I (calcium iodate), 0.5 mg; Co, 0.5 mg.

of chicks in each cage was calculated by the difference in their weight at the beginning and end of each period divided by the number of birds at the end of the period. The FCR corrected for it and expressed as grams of feed consumed in each cage divided by grams of WG (Imari et al., 2020). Feed intake (FI) in each phase was calculated by subtracting the remaining feed from the feed given to each cage during the phase. The chicks were fed *ad libitum* throughout the experiment. The relative humidity and the light programme of the rearing house were set at 60% and 18 h of light and 6 h of darkness, respectively.

2.2 | Tibia characteristics

To determine the morphological characteristics of the tibia, 6 birds were selected from each treatment on days 16, 19, and 23 (6 birds for each day). The chickens were weighed individually with a digital scale. All chickens were then euthanized by injection of pentobarbital (25 mg/kg BW) into the abdominal cavity. Tibia bones were collected to measure weight, length, median diameter, and growth plate width (GPW). In addition, the tibia weight index (tibia weight to bird's BW ratio, mg/g), GPW to TL ratio, and tibia median width to TL ratio were determined. Measurements were performed with calipers and a digital scale with a sensitivity of 1/1000 g. GPW was measured microscopically with DP2-BSW software, Olympus BX51 microscope and Olympus DP25 camera.

2.3 | Carcass analysis

At 42 days of age, one bird from each pen was selected, weighed, and decapitated to measure carcass traits. After peeling, thighs, breast, neck, back, and wings were weighed separately along with liver, gizzard and proventriculus, and evacuated digestive tract. Different parts of the gastrointestinal tract were separated and cut lengthwise with a scissor, washed with normal saline solution, dried with a towel and weighed. The dressing carcass was obtained from the total weight of the thighs, breast, neck, back, and wings after removing all the internal organs and oftentimes the head as well as inedible (or less desirable) portions. The results were expressed as the percentage of live weight (Imari et al., 2020).

After slaughter and weighing the carcass parts on 42 days, the left tibia was separated and soft tissues were detached. In order to separate the fat from tibias, they were immersed in ethyl alcohol for 48 h and again in diethyl ether $(C_2H_5)_2O$ for 48 h. The tibias were then dried for 24 h using an oven at 105°C until they reached a constant weight. Their ashes were then obtained using an electric furnace at 550°C for 12 h (Imari et al., 2020).

2.4 | Statistical analysis

The data were analyzed using the GLM procedure of SAS software (2012; Version 9.1) in a completely randomised design. Data were subjected to two-way analysis of variance in a 2 × 2 factorial arrangement with thiram and CS as the main effects. Significant differences of the means were separated by Tukey's test ($p < 0.05$).

3 | RESULTS

3.1 | Growth performance

The effects of CS and thiram administration on the growth performance of broilers are shown in Tables 3 and 4. Mean BW of chickens exposed to CS of 15°C at the age of 11–20 days and also birds that received thiram at a dose of 50 mg/kg diet during 11–14 days at 24 and 42 days of age, were significantly ($p < 0.01$) lower than NT and thiram0 groups, respectively. CS and thiram50 significantly decreased the WG of broilers during 11–24 days, 25–42 days and 11–42 days ($p < 0.05$). The interaction between house temperature and adding thiram to the diet was significant ($p < 0.01$) in the cases of BW at 24 and 42 days and also WG of birds during 11–24 days, 25–42 days and 11–42 days ($p < 0.01$). The mean BW of NT0 group at 24 days of age was significantly higher ($p < 0.01$) than NT50 and CS50 groups but it was not significantly different with CS0 treatment. The mean BW of chickens in treatment NT0 at 42 days of age was significantly higher ($p < 0.01$) than the other three groups. The mean WG in group NT0 was significantly higher ($p = 0.01$) than treatment CS50 during 11–24 days but it was not significantly different from NT50 and CS0 treatments. The mean WG of birds in group NT0 during the periods of 25–42 days and 11–42 days was significantly higher ($p0 < 0.01$) than the other three treatments (Table 3).

FI of broilers during 11–24 days and 11–42 days was not affected by thiram, rearing temperature and their interaction, but during 25–42 days FI in the Thiram50 group was significantly ($p < 0.05$) lower than Thiram0 group. During none of the periods, the interaction of rearing temperature and thiram had a significant effect on the FI (Table 4).

FCR in the CS group was significantly higher than the NT group during 25–42 days and 11–42 days of age ($p0 < 0.05$). During none of the periods, the main effect of thiram and the interaction of thiram with rearing temperature had a significant effect on FCR (Table 4).

Mortality of broilers during 11–42 days was significantly affected by CS, thiram, and their interaction ($p < 0.05$). Mortality in CS and thiram50 groups were significantly increased ($p < 0.05$) compared to NT and thiram0 groups, respectively. The interaction of house temperature and thiram addition to the diet had a significant effect on mortality. Adding thiram to the diet under CS conditions resulted in more mortality than adding thiram at NT conditions ($p < 0.05$; Table 4).

3.2 | Tibia characteristics

The tibia ash percentage was not affected ($p > 0.05$) by rearing temperature, adding thiram to the diet and their interaction (Table 5).

At day 16 of age, TW and TW to TL ratio in CS chicks were significantly higher ($p < 0.01$) than the NT group. TW in the thiram50 group was significantly ($p < 0.01$) higher than thiram0 group. The interactions of rearing temperature and thiram had a significant effect on TW and TW to TL ratio ($p < 0.01$). So that, the addition of thiram in CS conditions had a greater effect on the TW and the TW

TABLE 3 Effects of cold stress and thiram on body weight and body weight gain in broiler chickens*

Treatments		Body weight (g/bird) 24 days	Body weight (g/bird) 42 days	Weight gain (g/bird/day) 11–24 days	Weight gain (g/bird/day) 25–42 days	Weight gain (g/bird/day) 11–42 days
Main effects						
Temperature	Normal	856.3^a	2346.0^a	44.64^a	82.76^a	66.08^a
	Cold stress**	736.9^b	2110.4^b	38.70^b	76.31^b	59.58^b
SEM		28.58	44.36	2.17	1.58	1.42
p value		0.009	0.002	0.048	0.010	0.005
Thiram***	0	863.0^a	2336.3^a	46.08^a	81.85^a	66.21^a
	50 mg	730.2^b	2120.2^b	36.62^b	77.22^b	59.46^b
SEM		28.58	44.36	2.17	1.58	1.42
p value		0.005	0.003	0.007	0.05	0.004
Interaction effects						
Temperature × Thiram						
Normal	0	921.4^a	2493.8^a	49.32^a	87.36^a	70.72^a
	50 mg	791.3^b	2198.3^b	39.95^{ab}	78.17^b	61.45^b
Cold stress	0	804.6^{ab}	2178.8^b	42.84^{ab}	76.34^b	61.68^b
	50 mg	669.1^c	2042.0^b	33.29^b	76.28^b	57.47^b
SEM		40.43	62.74	3.07	2.23	2.002
p value		0.004	0.001	0.01	0.008	0.002

Note: $^{a–c}$Values within a column with different superscripts differ significantly ($p < 0.05$).

Abbreviations: SEM, standard error of the mean; TD, thiram-induced dyschondroplasia.

*Each value represents the mean of five replicates (20 birds per replicate).

**Cold stress was applied during 11–20 days at a constant temperature of 15 ± 1°C.

***Thiram was added to the diets at a dose of 50 mg/kg diet during 11–14 days to induce TD.

TABLE 4 Effects of cold stress and thiram on feed intake, feed conversion ratio and mortality in broiler chickens*

Treatments		Feed intake* (g/bird/day) 11–24 days	25–42 days	11–42 days	Feed conversion ratio* (g/g) 11–24 days	25–42 days	11–42 days	Mortality (%)
Main effects								
Temperature	Normal	106.0	152.2	132.0	2.58	1.84[b]	2.0[b]	5.50[b]
	Cold stress**	106.4	150.4	131.1	2.84	1.97[a]	2.2[a]	11.5[a]
SEM		4.47	1.53	3.41	0.265	0.042	0.065	2.57
p value		0.956	0.697	0.854	0.499	0.042	0.05	0.012
Thiram***	0	109.8	156.8[a]	136.2	2.42	1.92	2.07	7.0[b]
	50 mg	102.6	145.8[b]	126.9	3.01	1.89	2.15	10.0[a]
SEM		4.46	3.33	3.41	0.264	0.04	0.06	2.57
p value		0.267	0.034	0.071	0.137	0.592	0.42	0.042
Interaction effects								
Temperature × Thiram								
Normal	0	108.0	159.6	137.0	2.22	1.83	1.94	5.0[b]
	50 mg	104.0	144.9	127.1	2.95	1.85	2.08	6.0[b]
Cold stress	0	111.7	153.1	135.5	2.62	2.02	2.2	9.0[ab]
	50 mg	101.1	146.8	126.8	3.06	1.93	2.21	14.0[a]
SEM		6.32	4.71	4.82	0.374	0.06	0.09	2.55
p value		0.666	0.279	0.321	0.405	0.154	0.179	0.045

Note: [a,b]Values within a column with different superscripts differ significantly ($p < 0.05$).

Abbreviations: SEM, standard error of the mean; TD, thiram-induced dyschondroplasia.

*Each value represents the mean of five replicates (20 birds per replicate).

**Cold stress was applied during 11–20 days at a constant temperature of 15 ± 1°C.

***Thiram was added to the diets at a dose of 50 mg/kg diet during 11–14 days to induce TD.

to TL ratio reduction compared to normal conditions. House temperature, thiram and their interaction had no significant effects on TL, GPW, TWT, TWI and GPW/TL indices (Table 5).

At day 19 of age, TL in CS chicks was significantly ($p < 0.05$) lower than the NT group. The main effects of house temperature and thiram had no significant effect on TWI, TWT, GPW, TW, and GPW/TL and TW/TL ratios. The interactions of rearing temperature and thiram had a significant effect on TL, GPW and GPW/TL ratio. The addition of thiram under CS conditions decreased these three indices but increased them at NT conditions (Table 6).

At day 23 of age, the main effect of thiram had a significant effect on GPW. The value of this index in Thiram50 chicks was significantly higher than Thiram0 birds. The main effect of CS and the interaction of CS per thiram had no significant effect on this index. CS, thiram and their interaction had no significant effect on TL, TW, TWT, TWI, TW/TL and GPW/TL indices (Table 7).

3.3 | Carcass traits

Carcass traits and internal organs of broiler chicks, including dressing percentage, breast, thighs, evacuated intestines, liver, heart, total evacuated digestive tract, and evacuated gizzard and proventriculus, were not affected by CS, thiram and their interaction at 42 days of age ($p < 0.05$). Except that the interaction of rearing temperature and thiram affected the relative weight of gizzard and proventriculus ($p < 0.01$). The weight of these organs in the thiram receiving groups under CS conditions was significantly decreased compared to the groups that did not receive thiram ($p < 0.01$), but the weight of these organs was increased at NT. However, this increase was not significant (Table 8).

4 | DISCUSSION

4.1 | Growth performance

One purpose of the present study was to evaluate the effects of CS on the growth performance of broilers. In addition, the assessment of the ameliorative effects of cold conditioning on the induced TD by thiram. This study clearly showed that the growth performance of birds exposed to a period of CS was reduced compared to groups raised at NT. Similar to our study, the adverse effects of CS on WG and FCR have been reported by others (Rahmani et al., 2017). This

TABLE 5 Effects of cold stress and thiram on tibia bone properties in broiler chickens on 16 days of age*

Treatments		TL (mm)	TW (mm)	GPW (mm)	TWT (g)	TWI (mg/g)	GPW/TL (mm/mm)	TW/TL (mm/mm)	Tibia ash (%),42 days
Main effects									
Temperature	Normal	55.17	4.08[b]	3.216	6.31	0.017	0.056	0.074[b]	14.60
	Cold stress**	53.67	4.58[a]	3.15	6.14	0.0168	0.058	0.086[a]	15.21
SEM		0.980	0.111	0.075	0.358	0.001	0.002	0.002	1.09
p value		0.292	0.005	0.619	0.746	0.862	0.353	0.011	0.697
Thiram***	0	54.17	4.50[a]	3.168	6.092	0.015	0.056	0.083	15.83
	50 mg	54.67	4.17[b]	3.194	6.36	0.018	0.0585	0.077	13.98
SEM		0.980	0.111	0.075	0.358	0.001	0.002	0.002	1.08
p value		0.292	0.005	0.619	0.746	0.862	0.353	0.133	0.246
Interaction effects									
Temperature × Thiram									
Normal	0	54.5	4.17[b]	3.252	6.17	0.015	0.055	0.077[b]	16.14
	50 mg	55.83	4.0[b]	3.163	6.45	0.018	0.056	0.072[b]	13.06
Cold stress	0	53.83	4.83[a]	3.09	6.02	0.016	0.057	0.090[a]	15.52
	50 mg	53.5	4.33[b]	3.215	6.27	0.0182	0.006	0.082[ab]	14.90
SEM		1.38	0.158	0.112	0.507	0.001	0.002	0.004	1.21
p value		0.652	0.008	0.714	0.942	0.364	0.680	0.033	0.465

Note: [a,b]Values within a column with different superscripts differ significantly ($p < 0.05$).

Abbreviations: GPW, growth plate width; SEM, standard error of the mean; TL, Tibia length; TW, Tibia width; TWI, Tibia weight index; TWT, Tibia weight.

*Each value represents the mean of six replicates.

**Cold stress was applied during 11–20 days at a constant temperature of 15 ± 1°C.

***Thiram was added to the diets at a dose of 50 mg/kg diet during 11–14 days to induce TD.

result may be due to increased metabolism and the use of more nutrients to generate heat to maintain the body temperature of birds under CS (Ipek & Sahan, 2006) or due to the long duration of CS.

Ashraf et al. (2020) showed that birds exposed to CS had lower BW and feed efficiency (FE) than birds raised at NT. These authors also reported that broilers under CS have poor growth performance despite increased FI. Zhou et al. (2021) reported that CS significantly increases the FCR in young chickens, indicating a redistribution of nutrients from growth to regulating body temperature. In addition to poor growth performance, exposure to cold leads to defects in various physical activities, permanent tissue damage, and ultimately death in young broilers (Ashraf et al., 2020). In the present study, the mortality rate of chickens increased, which agrees with their results. Rahmani et al. (2017) reared broilers from the age of 14–42 days under chronic CS of 13–15°C and observed that WG and FE were decreased but FI was increased. These results agree with the results of the present study, except that feed consumption in our study did not show a significant change, which is probably due to the shorter duration of CS. Although chickens are warm-blooded animals and can cope with CS and balance body temperature, severe or sudden CS may be harmful to chickens (Borsoi et al., 2015). It has been reported that CS significantly increases FCR and reduces WG in young chickens (Yang et al., 2014). Our findings showed that CS increases FCR, which was consistent with previous studies, which suggest that redistribution of nutrients is from growth toward thermoregulatory responses.

Zhou et al. (2021) subjected 10-day-old broilers to a CS of 16 ± 1°C for 72 h. They found that this treatment significantly reduced the mean BW and increased the FCR of broilers, but FI was not affected by exposure to cold. In the present experiment, although the duration and severity of CS were higher (15 ± 1°C for 10 days), the FI of chickens was not affected. Su et al. (2020) raised broilers from 8 to 42 days of age in cold conditions, 3 or 12°C below the NT. They showed that the application of 34 days of CS at 3°C below NT had no adverse effect on growth performance. In addition, Su et al. (2020) showed that cold stimulation of 12°C below NT causes a response to CS and reduces broilers' growth and immunity. At the same time, it was found that CS of 3°C below NT was relatively mild for broilers. This CS not only did not adversely affect birds' growth performance but also decreased mortality compared to the control group (Su et al., 2020). Cândido et al. (2016) also showed that WG and FCR of chickens raised in mild CS conditions (27°C in

TABLE 6 Effect of cold stress and thiram on tibia bone properties in broiler chickens on 19 days of age*

Treatments		TL (mm)	TW (mm)	GPW (mm)	TWT (g)	TWI (mg/g)	GPW/TL (mm/mm)	TW/TL (mm/mm)
Main effects								
Temperature	Normal	65.0^{a}	5.167	3.91	8.75	0.016	0.061	0.079
	Cold stress**	59.75^{b}	5.33	3.56	7.81	0.016	0.058	0.089
SEM		1.99	0.232	0.169	0.42	0.646	0.002	0.003
p value		0.006	0.618	0.917	0.13	0.591	0.738	0.058
Thiram***	0	62.83	5.08	3.64	8.08	0.015	0.058	0.822
	50 mg	61.92	5.42	3.85	8.48	0.017	0.061	0.086
SEM		1.199	0.232	0.017	0.42	0.041	0.002	0.003
p value		0.595	0.323	0.383	0.521	0.646	0.438	0.393
Interaction effects								
Temperature × Thiram								
Normal	0	63.50^{ab}	4.83	3.413^{b}	7.83	0.014	0.051^{c}	0.076
	50 mg	66.50^{a}	5.5	4.504^{a}	9.67	0.018	0.069^{a}	0.083
Cold stress	0	60.33^{b}	5.33	3.86^{ab}	8.33	0.016	0.065^{ab}	0.089
	50 mg	59.17^{b}	5.33	3.20^{b}	7.28	0.019	0.053^{bc}	0.091
SEM		1.69	0.329	0.240	0.598	0.005	0.004	0.005
p value		0.026	0.525	0.008	0.061	0.914	0.012	0.206

Note: $^{a\text{-}c}$Values within a column with different superscripts differ significantly ($p < 0.05$).

Abbreviations: GPW, growth plate width; SEM, standard error of the mean; TL, Tibia length; TW, Tibia width; TWI, Tibia weight index; TWT, Tibia weight.

*Each value represents the mean of six replicates.

**Cold stress was applied during 11–20 days at a constant temperature of 15 ± 1°C.

***Thiram was added to the diets at a dose of 50 mg/kg diet during 11–14 days to induce TD.

the first week, 24°C in the second week and 21°C in the third week) from 1 to 21 days of age had no significant difference with chickens raised in NT conditions.

Mendes et al. (1997) noted that CS condition of 15.5°C caused a significant increase in FI, FCR and mortality during 3–6 weeks. Also, CS significantly reduced BW at 42 days, which is consistent with this experiment's results regarding FCR and mortality. However, unlike the present experiment, they observed an increase in FI, probably due to the older age of the chicks exposed to the cold because the older chicks are more compatible with CS. Renwick and Washburn (1982) showed that broilers raised at 26.7°C had higher FCR and mortality than those raised at 32.2°C, as well as poorer WG during the early ages. In our study, CS of 15°C significantly decreased WG and FE during 11–42 days of age and BW at 42 days but had no significant effect on FI, consistent with previous results (Nguyen et al., 2015; Zhou et al., 2021). The present investigation results showed that the CS studied in this study probably caused excessive stress in broilers and decreased growth performance.

However, the effect of CS on the growth performance of chickens is still controversial. Baarendse et al. (2006) observed that exposure to moderate CS (initial temperature of 28°C and 1°C decrease daily in a 5-day period) in the early posthatching period has long-term negative effects on chicks growth performance. However, Shinder et al. (2011) reported that exposure of broilers in the late embryonic stage to acute cold improves growth performance. These differences may be due to several factors, including experimental conditions, ambient temperature, age, chicken strain, and duration of CS (Shinder et al., 2011).

Nguyen et al. (2015) reared broilers in cold conditions. The temperature was initially set at 26.7°C and gradually decreased by 1°C per day until 19.7°C. Their data showed that chronic but mild CS improves the growth performance of chickens in the first week after hatching, market BW and FCR in the whole rearing period (Nguyen et al., 2015). Although FI remained unchanged, WG under chronic cold conditions was significantly increased compared to control chickens, resulting in significantly improved FCR.

Su et al. (2020) also reported that the growth performance of broilers reared at 12°C below NT is decreased compared to the control group. However, cold conditions below 3°C have no adverse effect on birds' growth performance. Comparing the results reported by these researchers with the present results showed that more severe CS (12°C) than 15°C used in the present experiment caused lower WG, FE and FI. However, in the present experiment, FI did not show a significant decrease. Research by Nguyen et al. (2015) showed that mild and chronic CS has no significant effect on broilers FI.

TABLE 7 Effects of cold stress and thiram on tibia bone properties in broiler chickens on 23 days of age*

Treatments		TL (mm)	TW (mm)	GPW (mm)	TWT (g)	TWI (mg/g)	GPW/TL (mm/mm)	TW/TL (mm/mm)
Main effects								
Temperature	Normal	67.83	5.92	4.03	11.24	0.017	0.055	0.087
	Cold stress**	69.08	5.92	4.25	11.42	0.017	0.061	0.086
SEM		1.21	0.232	0.163	0.545	0.80	0.003	0.002
p value		0.474	0.99	0.364	0.814	0.663	0.318	0.666
Thiram***	0	68.83	5.83	3.89^{b}	11.72	0.017	0.057	0.085
	50 mg	68.08	5.83	4.48^{a}	11.72	0.017	0.057	0.085
SEM		1.21	0.232	0.164	0.544	0.8	0.003	0.002
p value		0.666	0.618	0.028	0.332	0.772	0.718	0.356
Interaction effects								
Temperature × Thiram								
Normal	0	67.83	5.67	3.865	11.25	0.017	0.057	0.084
	50 mg	67.83	6.17	4.275	11.23	0.016	0.054	0.091
Cold stress	0	69.83	6.0	3.933	12.18	0.017	0.057	0.086
	50 mg	68.33	5.83	4.642	10.67	0.017	0.064	0.085
SEM		1.712	0.329	0.233	0.77	1.131	0.004	0.003
p value		0.821	0.736	0.098	0.583	0.888	0.468	0.534

Note: ^{a,b}Values within a column with different superscripts differ significantly ($p < 0.05$).

Abbreviations: GPW, growth plate width; SEM, standard error of the mean; TL, Tibia length; TW, Tibia width; TWI, Tibia weight index; TWT, Tibia weight.

*Each value represents the mean of six replicates.

**Cold stress was applied during 11–20 days at a constant temperature of 15 ± 1°C.

***Thiram was added to the diets at a dose of 50 mg/kg diet during 11–14 days to induce TD.

In this experiment, thiram at a dose of 50 mg/kg diet was added to the diet at 11–14 days of age to induce TD in chickens. Thiram significantly reduced the WG of chickens compared to groups that did not receive thiram. Although it decreased FI and FE, its effect was not significant. In agreement with the results of this experiment, Zhang et al. (2018) reported a reduced but nonsignificant FI in the thiram group compared to the control group. In the present study, our results confirmed a previous study that thiram reduced growth performance by inducing TD (Zhang et al., 2018). Recent studies have reported that TD reduces physical activity levels, lack of eating and drinking, difficulty standing and walking, and foot disorders that decrease WG (Mehmood et al., 2018).

4.2 | Tibia characteristics

TD is an important metabolic disorder that causes problems in the tibial cartilage of fast-growing birds and affects the proximal growth plate of the tibia (Nabi et al., 2016). This complication causes difficulties in movement and can reduce the profitability of broiler chickens production by up to 30% (Edwards JR, 2000). TD finally leads to dyskinesia and growth retardation of broilers (Li et al., 2007). However, due to the complexity of the pathogenesis, no specific drugs have been developed to prevent and treat TD. Since TD discovery, many factors have been studied to determine the exact cause of the disease and find a way to prevent it. In the present experiment, tibial bone indices (weight, length and width of tibia and its proximal GPW) were measured to determine whether thiram could cause TD in broilers (Huang et al., 2018; Mehmood et al., 2018) and also studied that whether CS can cope with this anomaly or not.

In the present study, the bone ash percentage in different experimental groups was not significantly different. In a study by Edwards JR (1985), bone ash levels were decreased in the thiram group. The results of the later study do not agree with the results of the current experiment, maybe due to these researchers reduced the calcium and phosphorus levels of the diets. Edwards JR (1985) reported that thiram did not decrease the bone ash of the birds fed with diets containing adequate calcium and phosphorus levels. Meanwhile, Edwards JR (1985) reported a significant correlation between bone ash and the incidence of TD. Huang et al. (2018) concluded that the body weight of TD affected chickens was higher than the control group but it was decreased at 10 and 14 days of age.

TABLE 8 Effects of cold stress and thiram on the relative weight of carcass parts and digestive organs (% of live body weight) in broilers on 42 days of age*

Treatments		Thighs	Breast	Heart	Liver	Gizzard + proventriculus	Intestines	Total digestive tract	Dressing percentage**
Main effects									
Temperature	Normal	19.4	25.31	0.63	2.35	2.35	3.70	6.04	64.92
	Cold stress***	19.7	24.03	0.68	2.39	2.25	3.51	5.76	64.75
SEM		0.474	0.619	0.321	0.566	0.417	0.652	0.693	1.2
p value		0.760	0.338	0.256	0.741	0.342	0.343	0.293	0.860
Thiram#	0	20.5	25.04	0.65	2.38	2.35	3.80	6.15	64.45
	50 mg	18.62	24.30	0.66	2.36	2.24	3.41	5.65	65.23
SEM		0.474	0.619	0.321	0.566	0.417	0.652	0.693	1.20
p value		0.067	0.579	0.889	0.868	0.279	0.057	0.069	0.396
Interaction effects									
Temperature × Thiram									
Normal	0	18.85	25.36	0.62	2.28	2.27^{ab}	3.95	6.22	64.25
	50 mg	19.96	25.26	0.65	2.41	2.42^{a}	3.44	5.86	65.58
Cold stress	0	18.39	24.71	0.69	2.48	2.43^{a}	3.65	6.08	64.64
	50 mg	21.02	23.34	0.67	2.31	2.07^{b}	3.37	5.44	64.87
SEM		0.671	0.876	0.143	0.253	0.187	0.292	0.31	0.535
p value		0.438	0.632	0.611	0.268	0.002	0.568	0.590	0.542

Note: ^{a,b}Values within a column with different superscripts differ significantly ($p < 0.05$).
Abbreviations: SEM, standard error of the mean; TD, thiram-induced dyschondroplasia.
*Each value represents the mean of five replicates.
**Carcasses were peeled.
***Cold stress was applied during 11–20 days at a constant temperature of 15 ± 1°C.
#Thiram was added to the diets at a dose of 50 mg/kg diet during 11–14 days to induce TD.

The results of matching the body weight of the birds with tibia parameters showed that WG was faster than tibial growth and vascularisation of hypertrophic region of the growth plate. In the present study, the thiram decreased WG of the birds during 11–24 days and bone indices also supported of TD induction at 16 days of age.

Mehmood et al. (2018) used thiram at a dose of 50 mg/kg diet during 4–7 days of age and measured tibial bone parameters including length, width, weight, and width of the growth plate at the ages of 7, 10, 14 and 18 days. They reported that these indices in the control group and the group treated with tetramethylpyrazine were higher than the TD group, but the difference between groups was not significant. A similar result was observed in the present experiment, although we tried to treat TD with CS.

Huang et al. (2018) found that the TW in TD chicks at seven days of age is higher than normal birds due to joint swelling. It was decreased at 10 and 14 days of age which is consistent with our study. Abdulkarimi et al. (2017) reported that the width of the tibia and femur decreases in the cold-exposed group. They indicated that possible causes include high blood calcium levels in the cold-exposed birds, resulting in reduced calcium deposition in bone structures in the cold-exposed birds. This result contradicts the results of this experiment at 16 days of age, but with increasing age, a significant difference in TW between the two groups disappeared.

Moraes et al. (2002) concluded that ambient temperature had no significant effect on the width of the tibia or femur. The increase in tibial bone length in chickens fed with a diet containing thiram may be due to swelling of the growth plate. Huang et al. (2018), added thiram at a dose of 50 mg/kg in 4–7 days, and reported that the length of the tibia bone at 7 days was longer due to the swollen joint and then was decreased on days 10 and 14 compared to the control group. Abdulkarimi et al. (2017) reported that the TL was similar in CS and control groups. However, the length of the femur in the CS group was shorter than the control group, which can be concluded that severer and longer CS reduces the growth performance and leads to leg problems in broilers. The reasons for changes in tibial bone width are also applicable to its ratio to bone length in the current experiment.

The results of several studies have shown that the width of the proximal growth plate in the tibia increases in the TD group (Huang et al., 2017; Huang et al., 2018; Mehmood et al., 2018). At 19 days of age, interaction of the house temperature and thiram showed that GPW in CS50 group was significantly lower than that of NT50 treatment.

Abdulkarimi et al. (2017) reported that CS reduces the length and width of the tibia bone but subsequently reduces the width of the growth plate. The interaction of temperature and thiram at the age of 19 d showed that GPW to TL ratio in chickens fed with thiram containing diets in NT conditions, significantly higher than cold-stressed birds fed with diets without thiram that indicates CS may have an ameliorative effect on TD, but more studies are needed in this area.

As far as the authors know, we are the first group to test the effect of CS on TD in broilers. Given that the 10-day CS used in this experiment decreased the growth performance of broiler chickens and increased mortality, it is suggested that milder CS be used to evaluate the effects of CS on TD in future studies.

4.3 | Carcass traits

Unfavourable weather conditions cause physiological stress and affect the growth performance of chickens. One of the objectives of the present study was to evaluate the response of broilers to CS and its effect on carcass traits. Birds' exposure to extreme thermal stresses alters the immune response and hematochemical parameters (Hangalapura et al., 2003). Carcass traits were not affected by treatments in this experiment. However, it was reported that the percentage of carcasses in chicks under CS (70.3%) decreased compared to chickens reared at NT (72%; Rahmani et al., 2017), which is contrary to our result. In the latter study, broilers were exposed to chronic CS of 13–15°C for 28 days, which was longer than the period used in our experiment. Smith (1993) reared broilers from 23 to 49 days of age at a constant temperature of 23.9°C or at alternating temperatures of 23.9 and 35°C. The total carcass weight of the birds at 23.9°C was higher than those grown at alternating temperatures, but there was no difference in the dressing percentage, which is consistent with the current result.

Subapriya et al. (2007) reported that the proventriculus from birds fed thiram showed focal hyperplastic changes in the mucosal epithelium, partial necrosis of the mucosa epithelium, a number of liquefied crypts, infiltration of lymphocytes into the laminar propria, and focal mucosal fibrosis. Some proventriculus glands showed necrosis in the second week. The 30 ppm, 60 ppm thiram groups showed partial to complete necrosis of the mucosal epithelium, infiltration of lymphocytes into the proximal laminar, willie elongation, and macular degeneration in the second week. In the fourth week, birds received 15 ppm thiram showed mucosal hyperplasia with necrosis, focal accumulation of mononuclear cells, and heterophilic infiltration of the proventriculus gland. The 30- to the 60-ppm group showed proventriculus inflammation and elongation or elongation of the villi with multifocal infiltration of mononuclear cells.

It has been reported that CS increases intestine weight and its absorption potential in ducklings and mice (Konarzewski & Diamond, 1995; Thomas et al., 1996). Subapriya et al. (2007) reported that thiram at a dose of 30 mg/kg diet increased gizzard weight due to increased tissue fibrosis. Aboghanima (2020) indicated that CS decreases the weight of carcass parts, including liver, breast muscle and thighs. In the present study, CS decreased the relative weight of gizzard and proventriculus.

5 | CONCLUSIONS

The results of this experiment showed that the CS used in this experiment reduces the WG and FE of broiler chickens. The addition of thiram to the diets induced TD in chickens, and CS reduced the severity of this complication in the tibia. CS and thiram had no significant effect on carcass traits.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this investigation are available upon reasonable request from the corresponding author (A. Hassanabadi).

ETHICS STATEMENT

The authors confirm that the journal's ethical policies have been complied with, as noted on the journal's author guidelines page, and have received approval from the appropriate ethics review committee. The authors acknowledge that they have followed EU standards for the protection of animals used for scientific purposes (and feed laws, if necessary).

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