animal

Earlier hatching time predisposes Cobb broiler chickens to tibial dyschondroplasia

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Fertile eggs from Cobb 500 broiler breeder hens were incubated to provide low starting egg shell temperatures (EST; 36.9°C to 37.3°C) which were gradually increased to 37.8°C during the first 7 to 15 days of incubation compared with eggs incubated with a constant EST of 37.8°C (standard conditions) over the first 18 days of incubation. Time of individual chick hatching (measured at 6 h intervals from 468 h of incubation), chick weight, chick length and yolk weight were measured at take-off and BW was measured at 7, 14, 28, 34 and 42 days of age. Male birds at 34 and 42 days of age were assessed for their ability to remain standing in a latency-to-lie test. At 34 and 42 days, male birds were examined for leg symmetry, foot pad dermatitis, hock bruising and scored (scale 0 to 4, where 0 = no lesion and 4 = lesions extending completely across the tibial growth plate) for tibial dyschondroplasia (TD) lesions. The lower EST profiles caused chicks to hatch later than those incubated under the standard EST profile. Chicks which hatched at ⩽498 h incubation grew faster over the first 7 days than those that hatched later. There were significantly more birds (only males were studied) that hatched from the lower EST profiles with TD scores of 0 and 1 and fewer with score 4 at 34 days than those hatched under the standard profile. Male birds at 34 days with TD lesions ⩾3 stood for significantly shorter times than males with TD scores ⩽2. Moreover, male birds at 34 and 42 days with TD lesion scores of ⩾3 hatched significantly earlier and grew significantly faster over the first 2 weeks of age than did male birds with TD scores ⩽2. It appears possible to decrease the severity and prevalence of TD in the Cobb 500 broiler by ensuring that the birds do not hatch before 498 h of incubation.

Keywords: incubation, chicken, bone development, dyschondroplasia

Implications

Tibial dyschondroplasia (TD) contributes to leg weakness in broilers and the reluctance to move may lead to damage to the birds from flock mates and to reduced feed intake. The incidence and severity of TD in Cobb 500 chicks may be reduced by ensuring that the incubation conditions of the fertile egg delay chick hatch time until after 498 h of incubation. Chicks that hatch before 498 h have a higher early growth rate (first 7 days), which may be a major determinant for the subsequent development of TD.

Introduction

TD remains an extremely common skeletal abnormality in commercial broiler chickens worldwide (Pines *et al.*, 2005; Dinev *et al.*, 2012; Genin *et al.*, 2012). TD is described as one of the most prevalent leg weakness conditions seen in

modern broilers (Yalçin *et al.*, 2007), and lesions have been found to be present in 30% of broiler flock (Crespo and Shivaprasad, 2011; Shahzad *et al.*, 2015). Accurate prevalence figures are not readily available as TD is mostly sub-clinical and does not result in huge culling rates (Crespo and Shivaprasad, 2011) and has a very variable incidence (Leach and Monsonego-Ornan, 2007), but it does contribute to general leg weakness expressed as reluctance to move. The latter effect can result in considerable concern and consequence for the broiler farmer under modern welfare-accredited production systems. Considerable economic losses have been attributed to TD (Pines *et al.*, 2005). Reluctance to move can result in damage to the bird from other birds walking over them (scratching possibly leading to cellulitis) or reduction in growth rate due to reduced access to feeders (Wideman, 2016).

TD is known as a common developmental disease in several avian species that is related to rapid growth, particularly in early life (Orth and Cook, 1994; Praul *et al.*, 2000; Leach and Monsonego-Ornan, 2007; Dan *et al.*, 2009).

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TD has similarities to mammalian osteochondrosis (Orth and Cook, 1994), including several skeletal diseases in humans and pigs involving described mutations in collagen and parathyroid hormone (Leach and Monsonego-Ornan, 2007). In TD, the usual procession in long bones of chondrocyte phenotypes through to a fully differentiated state within the resting zone of the epiphysis is disrupted (Farquharson and Jefferies, 2000). The chondrocytes in the hypertrophic zone of long bones do not reach their expected size, remaining small and rounded (Thorp, 2008) and their lifespan is increased (Farquharson and Jefferies, 2000; Leach and Monsonego-Ornan, 2007). This results in an accumulation of immature cartilage in the metaphysis (Orth and Cook, 1994) and is most notable in the proximal tibia. The collagen in this cartilage is abnormal, the molecules being highly cross-linked (Orth and Cook, 1994), which impairs the resorption of cartilage by osteoclasts and makes the cartilage resilient to vascular invasion (Farquharson and Jefferies, 2000).

TD is thus regarded as an abnormality of differentiation of chondrocytes. A simple solution to prevent the disease has not been found (Pines et al., 2005). Many factors have been incriminated in the occurrence of TD, including genetic predisposition, an inappropriate calcium : phosphorus ratio in feed (i.e. a relative excess of phosphorus), metabolic acidosis resulting from an electrolyte imbalance in feed (excess chloride over the sum of sodium and potassium ions), copper deficiency, excess dietary cysteine or homocysteine and some mycotoxins (Orth and Cook, 1994; Thorp, 2008). Experimentally, TD has been induced by thiram treatment but the mechanisms involved in these studies may differ from the condition observed in the field (Orth and Cook, 1994). The exact mechanism behind the development of TD remains unknown (Pines et al., 2005).

Some studies have examined the effects of varying conditions during egg incubation and subsequent skeletal health in meat chickens. Studies have looked at incubation temperatures which varied from the accepted ideal (Oviedo-Rondón and Wineland, 2012). Yalçin et al. (2007) increased the incidence of TD by increasing early incubation temperatures for broiler chicken eggs and demonstrated differences in chondrocyte differentiation with these temperature profiles. Their studies concluded that TD incidence is probably due to delayed heat-shock protein 90-driven chondrocyte differentiation (Yalçin et al., 2007; Genin et al., 2008 and 2012).

Differences in embryo development between the commonly used strains of meat chicken (Cobb and Ross) have been observed (Tona et al., 2010), essentially describing marked differences in incubation duration, embryonic metabolic rate and subsequent early chick growth rate. It was shown that Cobb developed faster in the first 4 to 5 days, whereas the Ross embryo grew more rapidly in the 2nd week of incubation. The Cobb line hatched earlier and demonstrated a higher BW at 7 days of age. Studies on incubation effects on subsequent leg and skeletal health need to take bird strain into account. There have been field studies which have observed significant differences in leg health between these meat chicken strains (Kestin et al., 1999; Knowles et al., 2008).

The present study followed from earlier findings in a meat chicken breeder line that indicated that lowered incubation temperatures were associated with better leg strength during growth (Groves and Muir, 2014). Three incubation profiles, which started with targeted egg shell temperatures (EST) below the accepted ideal, were then gradually increased to 37.8°C compared with a profile of a constant EST of 37.8°C using Cobb 500 meat chicken fertile eggs. The objective of the experiment was to evaluate the comparative outcomes of chick hatchability, time of hatching and the subsequent length of time the hatched chick spent in the incubator (sojourn), yolk weights at the time of removal from the hatcher, chick growth rates and subsequent leg strength, as measured by latency-to-lie (LTL) testing and leg abnormalities at 34 and 42 days across each of the incubation profiles. A high prevalence of TD occurred in this study and comparisons of TD severity across incubation profiles and early growth rates were obtained. This presented an opportunity to gain insights into the multifactorial nature of the aetiology of TD in broiler chickens.

Material and methods

Animal ethics
All experimental procedures were approved by the University of Sydney Animal Ethics Committee (protocol approval number N00/6-2013/2/6006) and were conducted under strict compliance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes as prepared by the National Health and Medical Research Council, 2013.

Fertile eggs
In all, 576 Cobb 500 meat chicken eggs were obtained from a commercial breeder farm from hens of ~34 weeks of age at the time the eggs were laid. The eggs were stored in a room held at 17°C for 6 days before incubation. The eggs were randomised and individually weighed and labelled with a pencil. Eggs were placed onto 16 incubation egg fillers (36 per filler). One egg close to the centre of each filler had a temperature sensor (Remote Intelligent Multisensors: TSIC 716 Advanced Sensor Technology; Netic Pty Limited, Ryde, NSW, Australia) placed within 2 mm of the equator of the egg and contact with the egg was maintained by the use of thermal conductive paste (silicone heat transfer compound; Unick Chemical Corp., Gimhae, Republic of Korea). The sensors were connected to a remote physical monitor (Uptime Devices, Ryde, NSW, Australia) and to a notebook computer where a software program (Net Sensor Man™; Netic Pty Limited) recorded temperatures continuously at 1 min intervals from days 1 to 18 of incubation; 10 h before the start of incubation, the temperature of the egg holding room was raised to 25°C to preheat the eggs.

Incubation
The eggs were placed into four small 288-egg-capacity incubators (E2A-Multiquip Pty Limited, Austral, NSW,

Australia), with 144 eggs in each. These incubators had forced ventilation, evaporative humidity provision, digital temperature control and automated turning. Timing of incubation was determined from the time of placement of the eggs in the incubator. The eggs were maintained at target EST for the first 18 days (432 h) of incubation (Supplementary Table S1). Each incubator was set to follow a pre-determined profile based on the EST as measured by the sensors attached to the eggs on each filler. Incubator 4 was selected for the control profile which aimed to maintain EST at 37.8°C from beginning of the incubation until transfer to the hatcher incubator at 18 days. Incubators 1, 2 and 3 were targeted to follow differing profiles beginning at a lower initial temperature and progressively increasing EST until 37.8°C was reached, as shown in Supplementary Table S1. The targeted ESTs were achieved by manually changing the set point of each incubator in response to the EST shown by the sensors attached to the eggs. This was monitored and adjusted two to four times daily, with increased frequency of attention after 9 days of incubation when the embryos began to generate heat. Eggs were turned through 90° every hour from days 0 to 18.

At 432 h (18 days) of incubation, all eggs were transferred to metal hatcher trays, each of which was divided into 60 individual hatching cells. Eggs were placed in their setter filler tray groups but these were randomised amongst the hatcher trays and the hatcher trays were placed in a random pattern into a single larger incubator (Aussieset™; Bellsouth Pty Limited, Narre Warren, Victoria, Australia). The hatcher incubator temperature was initially set at 37.4°C air temperature and thereafter progressively decreased to 36.9°C by 21 days and 12 h (516 h) of incubation with relative humidity starting at 60% and rising to 65% over the same time period.

Hatching procedures

After 468 h of total incubation, eggs were observed in the hatching tray and any cell with a hatched chick was recorded. Thereafter, hatched chicks were observed and newly emerged chicks recorded at 6 h intervals until 516 h of total incubation time.

At 516 h of incubation, chicks began to be removed from the hatching trays. The time at which each tray was removed from the incubator was recorded. From this the amount of time each chick spent inside the incubator after hatching (sojourn) could be estimated. To compare characteristics of chicks hatching at different times 20 chicks per incubator (five per filler tray) were selected at random from the hatcher trays and were weighed, had their length from beak to top of middle toenail measured (Hill, 2008) and were humanely euthanised. Their yolk sacs were then removed and weighed. Hatched chicks ($n = 402$) were weighed and given a numbered wing badge. Their toe webs were marked by making a small incision with a scalpel (sanitised in a 1 : 20 suspension of 4.8% chloroxylenol (Dettol™, Reckitt Benkiser, Slough, England, UK) between each chick) so as to identify them to incubator of origin in case of loss of a wing badge. Chicks were assigned randomly to six floor pens

providing an approximately equal distribution of chicks from each incubator in each pen. Each pen had a floor area of 9 m², contained new wood shavings as litter, was equipped with two tube feeders and nipple drinkers and held a total of 66 to 68 chicks (mean density of 6.4 birds/m²). The chicks were fed the same commercially obtained bagged broiler starter (0.7 kg/bird), grower (1.2 kg/bird) and finisher rations (Farmyard broiler feeds, Weston Feeds, Enfield, NSW, Australia). Feeds were assayed for CP, calcium, total phosphorus, sodium and potassium by George Weston Laboratories (Enfield, NSW, Australia) and results and available specifications are shown in Table 1. Birds were weighed individually at 7, 14, 28, 34 and 41 days. On day 34, five visibly male birds from each incubator group were selected from each pen (total of 30 birds per incubator) and subjected to a modified LTL test (Berg and Sanotra, 2003; Groves and Muir, 2013). Only males were studied at these ages as they have more locomotion issues than females (Oviedo-Rondón et al., 2009) and locomotion deteriorates as meat chickens age (Talaty et al., 2010; Toscana et al., 2013). In brief, the bird was placed in a large tub containing ~3 cm depth of tepid (30°C to 33°C) water and timed for a maximum of 5 min or until it sat down, after which the bird was removed, weighed and humanely euthanised. Each bird was then examined and scored for presence of detachment of the cartilage cap of the femoral head (DC), fragility of the femoral neck and TD lesions. Actual sex was determined at necropsy. DC was scored as normal, detached cartilage cap or fracture of the femoral neck when dislocated from the hip joint. TD was visualised after a coronal section was taken through the proximal tibia using an upward incision from below the procnemial ridge (Kaupp, 1918) approximately through the midline by a single operator. TD was scored as 0 (no cartilage plug), and as a score of 1 to 4 if the cartilage plug extended across the width of the epiphyseal plate by ⩽25%, 26% to 50%, 51% to 75% and >75%, respectively. Typical appearance of each score is shown in Supplementary Figure S1. This process was repeated (LTL test and BW) at day 42 on all remaining visibly male birds in each pen.

Statistical analysis

All statistical analyses used the computerised data analysis software system STATISTICA version 6.1 (StatSoft Inc., Tulsa, OK, USA) or Genstat version 16.2 (VSN International Ltd, Hemel Hempstead, UK).

Time of hatching and sojourn of the hatched chick in the incubator were compared across incubators using ANOVA. As birds were repeatedly weighed during growth, chick weight at take-off and at 7, 14, 28, 34 and 42 days were compared across early or late hatching groups of chicks and also across groups that developed High or Low TD scores at 34 or 42 days using the linear mixed model (Genstat) with weights (transformed to natural logarithms) as dependent variable and hatch time group or TD level as a fixed variable and incubator number and incubator tray position as random variables.

Hatchability data were compared using contingency table (χ^2) analysis. Eggs which failed to hatch were first

categorised as fertile or infertile. The hatch of fertile eggs (HOF%) was then calculated and each incubator's result compared with incubator 4 as the reference. Comparisons of embryonic losses were compared using the number of fertile eggs as the denominator. As embryo losses were very low, these were combined into categories of 'Early loss' (embryo death at the membrane or blood ring stage which occurs under 4 days of incubation), 'Mid loss' (embryos showing a large black eye but without visible feathers and under 8 cm in length, generally up to 10 days of incubation) and 'Late loss' (embryos with feathers and >8 cm in length, assessed by stretching the embryo and measuring from beak tip to the origin of the toenail of the middle digit as described by Hill, 2008).

For some analyses, TD scores were collapsed into categories of Low (scores of 0, 1 or 2) and High (scores of 3 or 4). Comparisons between the birds within each TD category across hatch and sojourn times were compared using Student's t test. TD category proportions for each incubator were compared using contingency table analysis.

As the LTL test was terminated at 5 min, the actual LTL time for birds still standing at termination could not be determined. Hence, LTL results in seconds were compared across appropriate groups using Kaplan–Meier survival analysis, with data for birds remaining standing at 300 s being censored. Comparisons between groups of birds in LTL were made using Gehan's Wilcoxon's test, where there were two groups compared or the χ^2 test for more than two groups.

Results

Actual EST recorded by sensors in each incubator are shown in Figure 1. Observed ESTs tracked close to target except for incubator 2, where on days 4 and 5 overnight EST rose to 38.5°C to 39°C. This was corrected each morning but the unexpected rise increased average EST for days 4 to 6 of embryonation for this incubator.

The analyses conducted on feeds acquired for this experiment (Table 1) revealed some variations from normally accepted levels of some nutrients. The assay of the starter ration indicated low sodium and total phosphorus levels. The assays of the grower ration revealed higher calcium and total phosphorus levels than would be normally desired. Unfortunately, the formulation of this commercial bagged feed was unavailable. Adjusting the total phosphorus levels found on assay (assuming a typical ration would contain 2700 mg/kg phytate phosphorus; Selle P., personal communication) estimated that possible non-phytate phosphorus levels would be regarded as low in the starter feed and excessive in the grower ration.

Table 2 summarises hatchability, HOF% and embryo loss as a percentage of fertile eggs from each incubator. The differences in hatchability between incubators was not significant; however, when corrected for fertility of the eggs placed in each machine, incubator 1 had significantly higher HOF% compared with incubator 4 with incubators 2 and 3 intermediate. The higher HOF results were mainly due to
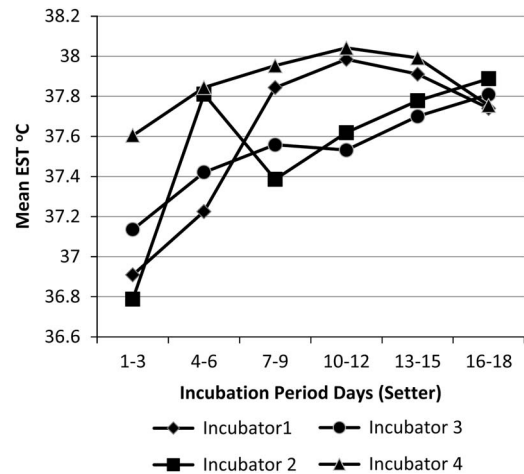

Figure 1 Mean egg shell temperatures (EST) recorded by temperature sensors attached to eggs in each incubator averaged over 3-day period of incubation.

Table 1 *Feed nutrient and mineral assay results*

Ration	Starter	Grower	Finisher 1	Finisher 2
CP (g/100 g)	21.6	21.0	19.7	18.3
Calcium (mg/kg)	7620	12 200	8660	9030
Phosphorus (mg/kg)	5810	9100	6000	6920
Sodium (mg/kg)	884	1690	1430	1300
Potassium (mg/kg)	8880	7850	9070	8990
Chloride (mg/kg)	1839	2204	2157	2084
DEB[1] (mEq/kg)	214	213	234	228
Ca : total P ratio[2]	1.31	1.34	1.44	1.30

[1]Dietary electrolyte balance (DEB) based on Mongin's equation (Na + K − Cl; after Thorp, 2008).
[2]Calcium : total phosphorus ratio calculated from assay results.

lower late losses (Table 2). There may have been a slight rise in mid period losses in incubator 2 possibly associated with the unintended high temperatures experienced on days 4 and 5, but the higher embryo loss here was minor and not enough to reach significance. Use of the lower EST profiles did not deleteriously affect hatchability.

The spread of hatch times in each incubator is depicted in Figure 2. The mean hatch time for eggs under the lower early EST profiles used in incubators 1, 2 and 3 was extended by 9 to 10 h compared with the standard EST profile used in incubator 4 (statistically significant at $P < 0.05$, as shown in Table 3).

There were no significant differences in hatch times between incubators 1, 2 and 3, but there was still considerable overlap of the hatch times of these incubators with that of incubator 4 kept at a constant 37.8°C EST profile. The distribution of chick hatch time in the lower EST incubators appeared to be biphasic as can be seen in Figure 2. An investigation on this aspect revealed that the majority of chicks which hatched earlier under the lower EST profile conditions came from the rear of the egg trays in those incubators. There appeared to be a stratification of

Table 2 *Hatchability and breakdown of embryo loss in each incubator as a percentage of fertile eggs*

| Incubator	1	2	3	4	Overall mean	SE (%)
Chicks hatched[1] (%)	89.51	90.38	82.01	81.25	85.79	1.455
Infertile (%)	2.10	0.69	3.60	2.78	2.28	0.622
HOF[2] (%)	91.43^a	90.91^{ab}	85.07^{ab}	83.57^b	87.79	1.380
Early loss[3] (%)	4.28	4.20	8.96	5.71	5.75	0.981
Mid loss[4] (%)	0.71	2.80	0.75	1.43	1.44	0.503
Late loss[5] (%)	4.29^{ab}	2.10^b	5.22^{ab}	9.29^a	5.21	0.937

[1]Total hatch as a percentage of all eggs set.
[2]Hatch of fertile (HOF = percentage of fertile eggs which hatched).
[3]Embryonic development limited to membrane or blood ring stages as a percentage of fertile eggs.
[4]Embryos with a large eye but without feathers and <8 cm length lost as a percentage of fertile eggs.
[5]Feathered embryos >8 cm lost as a percentage of fertile eggs.
^{a,b}Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$, χ^2 analysis).
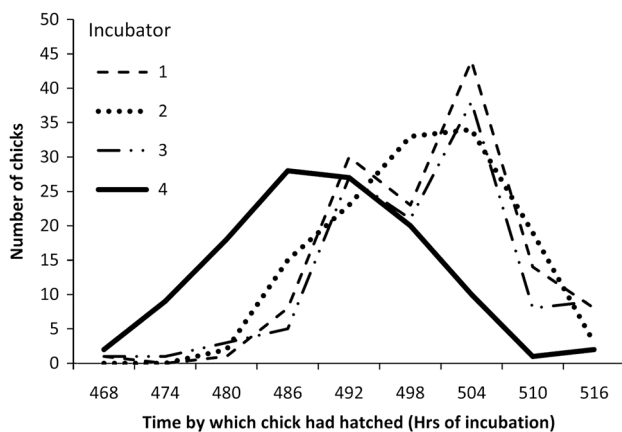
Figure 2 Distribution of chick hatch times in 6 h intervals by incubator.

temperatures across the incubators from front to rear (warmer towards the rear) when run at these lower temperatures.

Mortality during the experiment was low and with no significant difference between incubators with losses being 4.63%, 4.59%, 7.53% and 7.44% for chicks from incubators 1, 2, 3 and 4, respectively ($P = 0.38$ for the largest difference by χ^2 analysis). Measurements taken on a sample of chicks at take-off (from 516 h of incubation) from the incubator are shown in Supplementary Table S2. There were no significant differences in chick weight, chick length or yolk weight at take-off between incubators. However, when the actual time of hatch of chicks within each incubator was considered, chicks which had hatched by 498 h of total incubation (defined as 'Early') weighed significantly less than those which hatched after this time ('Late') by an average of 2.2 g/chick. The Early hatched chicks also were longer (by 0.3 cm) and had yolk sacs of lower weight (by 1.06 g) compared with Late hatched chicks.

The progressive weights of all Early and Late hatched chicks after hatching are shown in Table 4. Early chicks weighed less at take-off time but gained significantly more weight by day 7 than the Late hatched group. Thereafter, there was no significant difference between weights of the Early and Late groups.

Supplementary Figure S2 compares the ability of chicks hatched from each incubator to remain standing (LTL) at 34 days. There was no significant difference discernible between incubators for LTL at this time.

Table 5 shows the numbers of birds exhibiting leg abnormalities among those examined at the time of the 34-day LTL test. The lesions of foot pad dermatitis and hock burn were minor in severity but could be observed on a proportion of birds. There were no differences in prevalence of these lesions between incubation groups. Approximately 15% to 22% of birds exhibited a moderate lateral deviation of the shank from a straight alignment with the proximal leg ($\leqslant 2$ cm from the midline), but there were no significant differences in prevalence between the incubation groups. Birds in this experiment exhibited a high prevalence (42.3%) of TD lesions when examined at 34 and 42 days (displayed as prevalence of each lesion score by each incubator in Table 5) and 15.8% of all birds had TD scores exceeding score 2. Birds from the standard incubation programme (incubator 4) had significantly lower prevalence of birds with TD lesion score 0 than each of the slow incubation profiles ($P < 0.05$) and numerically higher prevalence of TD scores of 1 and 4 than the slow programme incubated birds.

Comparisons of the LTL outcomes for birds with different TD lesion scores were then conducted and it was apparent that the birds with TD scores of 3 or 4 (defined as 'High') stood for significantly ($P = 0.004$) less time than did those with scores of 0, 1 or 2 (defined as 'Low') (Figure 3).

After this outcome, further comparisons were made using the Low or High TD score as a grouping to develop more information on the birds with more advanced and severe TD lesions (Table 6). Chicks which developed High TD scores hatched 5 h earlier on average and hence spent an average of 4.5 more hours as hatchlings inside the incubator before take-off (sojourn), but did not differ in BW at take-off compared with chicks which exhibited Low TD scores. However, the chicks that would later show High TD scores (at 34 or 42 days) had significantly higher BW at 7 days of age compared with birds that had Low TD scores.

Table 3 *Mean hours of incubation time for chicks to hatch for each incubator*

Incubator profile	1 – Slow	2 – Slow	3 – Slow	4 – Standard[1]
Number of chicks hatched from 144 eggs	129	129	113	117
Mean time of hatch (h)[2]	499.8[a]	498.9[a]	499.3[a]	489.5[b]
SEM	0.74	0.72	0.86	0.88
95% Confidence interval (h)	498.4 to 501.3	497.4 to 500.4	497.7 to 500.9	487.9 to 491.0

[1]Egg shell temperature target of 37.8°C.
[2]Mean time to hatching determined at 6 h intervals from 468 h of incubation.
[a,b]Mean values with unlike superscript letters were significantly different ($P < 0.05$, ANOVA).

Table 4 *Measurements of birds which hatched Early or Late from take-off to 41 days*

	Mean (SE) of Early[1] hatched group	n[2] of Early hatched group	Mean (SE) of Late[3] hatched group	n[2] of Late hatched group
Sampled[4] chick BW (g)	39.2[B]		41.4[A]	
Sampled chick length (cm)	18.8[a]	44	18.5[b]	35
Sampled chick yolk weight (g)	3.65[B]		4.71[A]	
BW (g) at day 0 (take-off)	40.5[b] (0.29)	294	42.4[a] (0.37)	190
BW (g) at day 7	163[a] (1.26)	244	154[b] (1.45)	154
BW (g) at day 14	460[a] (3.58)	242	446[b] (4.25)	151
BW (g) at day 28	1527 (11.9)	240	1533 (14.7)	150
BW (g) at day 41	2639 (24.1)	164	2652 (31.0)	96

[1]Chicks hatched at $\leqslant$ 498 h of incubation.
[2]Number of birds weighed at specified age in each group. Day 0 weights include birds removed and sampled after take-off.
[3]Chicks hatched at >498 h of incubation.
[4]Chicks sampled at take-off.
[A,B]Mean values with unlike superscript letters at the same age were significantly different ($P < 0.01$).
[a,b]Mean values with unlike superscript letters at the same age were significantly different ($P < 0.05$).

Table 5 *Prevalence of leg abnormalities in birds examined at 34 days*

Leg lesions	Incubator 1 – slow	Incubator 2 – slow	Incubator 3 – slow	Incubator 4 – standard	SE (%)
Detached cartilage[1] (%)	43.9	55.8	54.4	55.3	3.51
Hock bruise[2] (%)	50.0	50.0	52.9	70.6	5.37
Foot pad dermatitis[2] (%)	29.8	26.4	43.5	31.9	3.29
Legs deviate[3] from straight (%)	15.8	22.6	19.6	14.9	2.71
TD score (0%)	65.5[a]	55.2[a]	67.4[a]	42.6[b]	3.44
TD score (1%)	10.3[ab]	17.0[ab]	8.7[b]	23.4[a]	2.48
TD score (2%)	10.3	9.4	13.0	8.5	2.13
TD score (3%)	10.3	3.8	6.5	8.5	1.58
TD score (4%)	10.3[ab]	9.4[ab]	4.3[b]	17.0[a]	2.13

TD = tibial dyschondroplasia.
[1]On disarticulation of the coxofemoral joint, the cartilage cap of the head of one or both of the femurs detached.
[2]Mild lesions only observed.
[3]Shank deviated from a straight alignment with the leg by up to 2 cm laterally at the metatarsophalangeal joint.
[a,b]Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).

Discussion

Early embryonic development is crucial to chondrocyte differentiation and this has been linked to the subsequent incidence of TD (Yalçin *et al.*, 2007; Genin *et al.*, 2008). Early variations in incubation temperatures were found to affect collagen gene expression that resulted in a wider proliferative zone of the tibial growth plate and this appeared to be more evident with lower early temperatures (36.9°C for 6 h/day from days 0 to 8 of incubation) using the Ross meat chicken strain. A study (Shim and Pesti, 2011) using Cobb broiler eggs showed that higher early incubation temperatures lead to earlier hatching and suggested that the ensuing longer sojourn of the early hatchlings in the incubator and prolonged times to placement of the chick may explain observed effects on bone development including TD.

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Other research (reviewed by Oviedo-Rondón and Wineland, 2012) has identified early and late embryo development stages as crucial for bone development and that generally the use of low or high early temperatures at these times can be associated with problems in bone development. The variety of experimental protocols used (incubation temperatures tested, the variation in the duration of the test temperatures, measurement of either incubator air temperature or EST, differences between broiler breeds used in different studies and different leg strength parameters) complicates an overall assessment of the effects of incubation conditions on bone development. Yalçin *et al.* (2007) were able to induce TD by applying variations to early incubation temperatures (elevated or decreased for 6 h/day

over days 0 to 8 of incubation). Those authors attributed the effect to this daily temperature variation. In contrast, the present study used a different broiler strain (with a known difference in incubation trajectory; Tona *et al.*, 2010) and employed lower early incubation temperatures which were consistent on a daily basis and were increased gradually over time. This was chosen because many studies in our laboratory studying lower early EST profiles (Groves and Muir, 2014) have demonstrated consistent improvement in chick bone strength over the accepted standard of continuous EST of 37.8°C.

Despite efforts made by the breeding companies to improve this condition (Davies, 2015), TD remains a common finding on routine *postmortem* examinations in contemporary commercial broiler flocks under Australian conditions (Groves P., personal observation). The heritability of leg problems, including TD, has been shown to be weak and it is apparent that genetic improvement will not be as rapidly effective as management measures in alleviating broiler leg weaknesses (González-Cerón *et al.*, 2015).

There is suggestive information regarding the contribution of incubation to the syndrome (Yalçin *et al.*, 2007; Shim and Pesti, 2011) but there are also many studies showing links to nutritional factors of the chick involving calcium, phosphorus, sodium, potassium, chloride and trace elements (Rennie *et al.*, 1993; Praul *et al.*, 2000; Genin *et al.*, 2008; Thorp, 2008; Oviedo-Rondón and Wineland, 2012). A recent study has shown the effect of high incubation temperature on chick growth plate development (Oznurlu *et al.*, 2016), which may thus have implications for developmental bone diseases such as TD. Early chick growth rate has also been strongly implicated (Orth and Cook, 1994; Leach and Monsonego-Ornan, 2007) in the development of TD. As summarised by Leach and Monsonego-Ornan (2007), TD is primarily a condition associated with rapid growth rate but is also responsive to potential insults to the epiphyseal growth
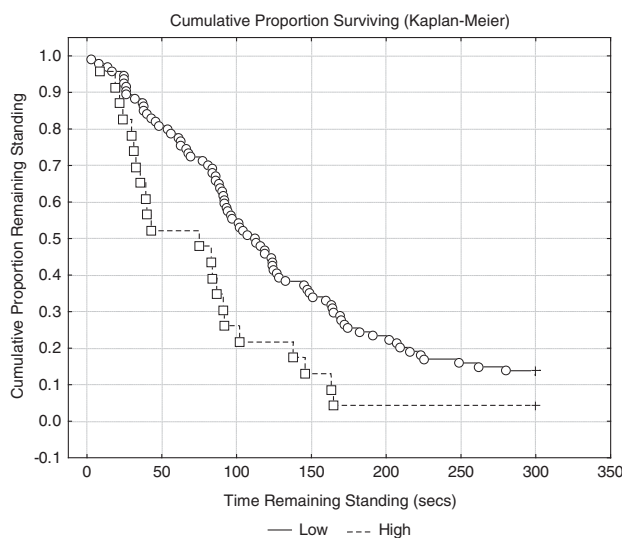

Figure 3 Latency-to-lie comparison for male birds with Low tibial dyschondroplasia (TD) scores (○: 0, 1 or 2) compared with birds with High TD scores (□: 3 or 4) at 34 days. (Censored cases are marked with +.) Gehan's Wilcoxon's test. Test statistic = 2.85; $P = 0.004$.

Table 6 *Hatch time, sojourn time and subsequent mean live weights of male birds examined for tibial dyschondroplasia (TD) lesions at 34 or 42 days*

Measurement	Mean (SE) for male birds with Low[1] TD lesions	*n*	Mean (SE) for male birds with High[2] TD lesions	*n*
Hatch time[3] (h)	499[a] (0.79)	141	494[b] (2.13)	29
Sojourn time[4] (h)	20.0[b] (0.80)	141	24.6[a] (2.12)	29
BW (g) at take-off[5]	41.6 (0.32)	141	41.6 (0.70)	29
BW (g) at day 7	159[b] (1.21)	141	170[a] (2.86)	29
BW (g) at day 14	468 (3.54)	141	477 (8.19)	28
BW (g) at day 28	1652 (12.51)	141	1654 (27.89)	29
BW (g) at day 34	2206 (21.73)	85	2186 (42.33)	22
BW (g) at day 42	3128 (37.62)	56	3102 (115.01)	6

n = Number of birds in the preceding column examined at 34 and 42 days.
[1]TD lesion scores of 0, 1 or 2 at day 34 or 42.
[2]TD lesion scores of 3 or 4 at day 34 or 42.
[3]Mean time to hatching determined at 6 h intervals from 468 h of incubation.
[4]Estimated minimum time chick remained in the incubator after hatching.
[5]Weight of chick at the time when it was taken out of the incubator.
[a,b]Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).

plate. However, as the occurrence of the condition varies markedly in the commercial field, where growth rate is invariably high, its occurrence would appear to require a multiple coincidental occurrence of factors to determine its clinical expression.

In the present study, chicks which emerged from the egg before 498 h of incubation lost weight during their subsequent sojourn in the hatcher before their removal from the incubator. This weight loss was accounted for predominantly by reduction in size of the yolk sac, and the chicks still grew in length during the sojourn without access to feed. Although these chicks weighed less at take-off than those that hatched after 498 h, they gained weight faster in the first 7 days after take-off compared with that of the Late hatching chicks. This was a short-term effect and growth rates after 7 days were equivalent with regard to hatching time and sojourn.

At 34 to 42 days of age, more often chicks that had hatched from the incubators in the slow incubation profile had TD scores of 0 and fewer birds had a score of 4, compared with those that had been incubated under a standard EST profile.

LTL outcomes have been shown to be shorter for birds with lameness (Hothersall et al., 2013). In the present study, birds that exhibited higher TD scores stood for a shorter time in an LTL test indicating that more severe TD lesions are associated with poorer leg strength and mobility.

The ability to discern significant differences between the standard and slow incubators resulted from the overlap in hatching times between these two groupings. This was due to a detected stratification of temperatures from front to back of the machines (warmer at the rear). Thus, chicks incubated closer to the rear of the slow incubators had similar hatch times to those incubated towards the front of the standard incubator. Analysis was still possible however when compared across actual chick hatching and sojourn times.

In the present study, significant associations were observed where Cobb birds with higher TD scores had hatched earlier and also grew faster in the first 7 days of life. This is in broad agreement with other reviews noting that rapid early growth is involved in the development of TD (Orth and Cook, 1994; Praul et al., 2000; Leach and Monsonego-Ornan, 2007). These findings are also in agreement with Shim and Pesti (2011), where increased leg weaknesses were associated with a longer sojourn of the chick in the hatcher. Shim and Pesti (2011) hypothesised that this observation was due to some level of dehydration. Our findings would indicate that, in the Cobb broiler, the effect of early hatching and thus a longer sojourn on subsequent TD development would be more related to the enhanced early chick growth rate. Hatching time and sojourn were strongly correlated in this experiment (Spearman's rank $R = -0.98$). This experiment was not designed to separate the effects of hatch time from sojourn and this will be examined in further studies. Other studies (unpublished) have shown that this accelerated early chick growth does not occur if the earlier hatched chicks are removed from the incubator and given access to feed promptly. Hence, it is suspected that the possible effect is primarily related to extended sojourn but this needs to be confirmed in purpose designed experiments. Hatch times however were significantly altered by the incubation temperatures employed.

The elevated prevalence of TD lesions observed in this experiment may have a relationship with the rations the chicks were fed. Formulation of the commercially purchased bagged feeds was not available to us; however, nutrient analyses were conducted. Thorp (2008) suggests that a dietary electrolyte balance (DEB = Na+K−Cl) of >200 mEq/kg of feed is adequate to avoid TD development. The feed assay results in this study would indicate that the rations used met this DEB requirement. The low phosphorus level in the starter feed and the elevated calcium and phosphorus levels revealed in the grower feed could have contributed to the high prevalence of TD observed in the birds. With the definitive causation of TD still remaining unknown, the feed formulation may not have been the only predisposing factor for the incidence of TD observed in this experiment. Under the conditions of the present study, hatching of the Cobb chicks at a mean of 494 h of incubation (Table 6) resulted in a more rapid growth rate during the first 14 days of life. This higher early growth rate was significantly associated with a higher incidence and severity of TD lesions than that observed in chicks hatched at a mean of 498 h.

TD in the field is not always associated with gross lameness (unless extremely severe) but does cause reluctance to move. It is not commonly recorded as a common reason for culling. As Wideman (2016) points out, commercially grown meat chickens may conceal manifest symptoms of lameness to avoid persecution by other birds. Hence, birds with what may be considerable TD lesions may not appear as overtly lame and would not result in culling by the farmer. This does not reduce the welfare or production costs of TD; however, as birds may still be in pain and be reluctant to move and hence have reduced access to feed and water and may be walked over more frequently by their peers. Where TD is found as a problem in the field, it may be possible to decrease the severity and prevalence of the problem by adjusting incubation practices by lowering early EST to avoid early hatching of chicks or an extended sojourn. The use of a lower starting EST target for Cobb 500 broiler eggs did not adversely affect hatchability or chick quality.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S1751731116001105

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