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Broiler chicken skeletal integrity and incubation

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Effects of Incubation differences on Broiler chicken skeletal integrity.
Project No. ***09-24***

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Executive Summary

Skeletal weakness is a major concern in modern broiler chicken production. This has been shown to vary with broiler breed (Kestin *et al*., 1992), but in the field we see the same syndromes in both major breeds, only the level of occurrence may differ. An observation from a number of hatches of small numbers of breeder flocks disclosed a variation in the extent of an early skeletal problem between hatches and there were definable differences in the histories of incubation condition between these hatches. This encouraged us to attempt to recreate the syndrome by mimicking these conditions compared with an ideal incubation profile.

Fertile eggs from a line of broiler suspected of being very susceptible to skeletal problems were obtained and were randomised between two small incubators at the University of Sydney, one set to run under ideal conditions of temperature and humidity, the other following the putative conditions involved with expression of the skeletal problem. Climatic data loggers were placed with the eggs to verify the actual conditions experienced during incubation. At hatch, chicks from each incubator were randomly selected and sampled for bone ash and serum electrolytes. The remaining chicks were grown out to 42 days on a commercial broiler ration. At 14 days of age, randomly selected birds from each group were sampled for femoral bone ash and their tibias were sectioned and their epiphyseal growth plate width measured in a standard manner. At 28 days, more birds were randomly selected, sampled for serum electrolytes and their tibias examined for visible lesions of Tibial Dyschondroplasia (TD). At 42 days, randomly selected birds from each group were assessed for mobility using the "Latency To Lie" (LTL) technique (Weeks *et a*l., 2002). This technique has a high correlation to traditional "gait scoring" techniques but is more objective.

After the early results of study 1 were available, a second study was conducted using broiler eggs incubated in the same way, this time swapping the allocation of profiles to each incubator. Chicks hatched from the latter study were weighed, measured for beak to toe length and then sampled for serum electrolytes and bone ash. The limitation of the time span for this project did not allow us time to grow out the birds from study 2.

One of the incubators used malfunctioned in the first study (fortunately this was the control machine and the profile experienced by the test machine was close to that intended). Despite this, we were able to demonstrate significant differences in skeletal parameters in association with differing incubation conditions. Chick from the test incubation profile had lower bone ash and higher serum Ca and P at hatch. This would be consistent with an unbalanced effect of parathyroid hormone in these embryos. By 14 days of age, chicks from the test profile had wider tibial growth plates and lower femoral bone ash than the control group. There was a moderately strong negative correlation between bone ash % and growth plate width. This is possibly consistent with a sub-clinical incidence of a rickets-like condition. At 28 days, there was a relatively high incidence of TD in the birds but this did not differ between the two incubation groups. At 42 days, birds from the test group had significantly shorter latency to lie times than the controls. This test involves placing the birds in 3cm of tepid water and timing how long before they make an attempt to sit. Birds which have shorter LTL times are judged to be experiencing some degree of pain from continuous standing and LTL time is strongly correlated to standard gait scores.

Despite repairs to the problem incubator, this machine malfunctioned again during study 2 and hence results were not meaningful for the intention, although some interesting findings were discovered.

In conclusion, we were able to demonstrate effects on skeletal integrity from different incubation temperature profiles. This has connotations for the industry in understanding and improving the leg strength of broiler chickens under commercial conditions. Although incubation conditions as trialled here may not commonly occur throughout an entire commercial incubator, regional variations often occur within machines (hot and cool spots which may vary with ventilation patterns, egg size, proportion of viable eggs producing heat, etc). A better understanding of how these factors impinge on embryonic bone development may enable the industry to minimise the clinical expression of bone weakness for susceptible breeds in the field.

Contents

Introduction

"Leg weakness" problems in broiler chickens cause considerable loss to the industry and are regarded as a major welfare issue for this class of bird. The major cause of field mortality in broilers currently is cellulitis, mostly as a result of birds scratching each other. This scratching problem is exacerbated by lameness, as the lame birds cannot readily move out of the way of their shed mates. Causes of lameness include varus-valgus deformity, rotation of the tibiotarsus, tibial dyschondroplasia (TD) and femoral head necrosis (AAAP, 2006). These conditions have been prevalent for some time, however, their incidence has increased in recent years. We have also observed the occurrence of a bone mineralisation defect in newly hatched chicks, clinically appearing as "spraddle leg" (Figure 1), an old condition described as due to slippery hatchery trays. This description does not fit the current syndrome as these chicks have extremely weak bones – almost rachitic in clinical appearance. Affected chicks almost invariably have abrasive lesions on the posterior skin surface of the hock joints (Figure 2) most likely from extended squatting or walking on this area. This is an emerging and serious problem. These weak boned chicks commonly account for 50% of the first week chick mortality (due to non-starters that can't effectively feed – these commonly have a small abrasion on both hocks indicating hock-walking, and their bones are soft). Culling due to leg weakness through many flocks can reach 4%.

Recent communications have implicated defects in incubation as possible contributors to some bone irregularities. Spraddle legs have been associated with high humidity during incubation (Crespo & Shivaprasad, 2008) and Genin *et al.* (2008) implicated cyclic overheating during the first 8 days of incubation in the later incidence of TD via an effect on growth plate hypoxia. In the field, rotated tibia is becoming one of the major leg deformities seen. The aetiology of this condition is not known but early rickets may be a predisposing factor (Crespo & Shivaprasad, 2008). Thorp (2008) also implicates earlier occurrence of rickets or dyschondroplasia with varus-valgus deformity.

Clinically we see this young chick bone weakness at varying intensities, more often with young donor flocks. But the incidence occasionally can be unexpectedly high and variable between hatches of eggs from a number of breeder flocks. These conditions have also appeared to be non-responsive to heavy vitamin D supplementation of the broilers and nutrition of the breeder flocks cannot be faulted. An effect from incubation irregularities appears as a candidate for a part in the aetiology of this group of conditions.

As a pre-emptive factor to this research project, chicks from a small Grandparent hatchery were recently observed to experience an inordinately high incidence of spraddle legs and weak bones in two hatches but not in other contemporaneous flocks, and this was related to one incubator with some records of its conditions. In this instance, temperatures over days 5-7 were variable and humidity in early incubation was low. Therefore we proposed to replicate this incubation pattern experimentally and to examine its effects on the skeletal integrity of the chicks produced. We hypothesised that these later leg deformities, including rotation of the tibia and general leg weakness, may be linked to the occurrence of the early bone weakness seen at hatch and was assessed over a 6-week grow out of chicks hatched under varied incubation conditions.

Figure 1. Spraddle or Splay leg chick

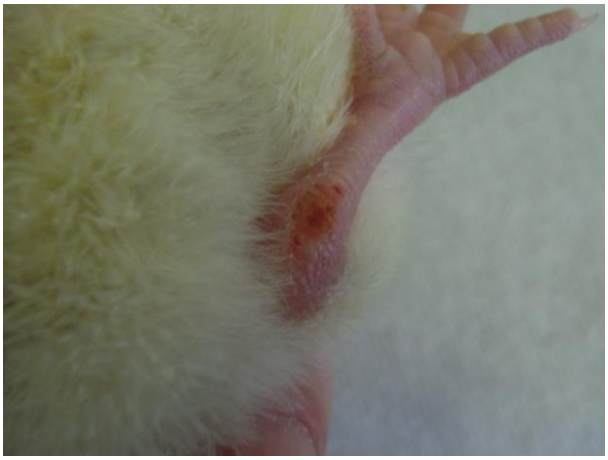

Figure 2. Abrasive lesion on hock joint area

Objectives

The objective of this project was to determine whether variation in incubation conditions generated a higher incidence of early bone weakness in newly hatched chicks and to evaluate if later skeletal deformities or leg weakness could be associated with the incubation profile.

Methodology

Two incubator studies have been run, with chicks from study one been grown out until 6 weeks of age, whereas in study 2 day of hatch observations were made prior to all chicks being euthanized. General methodology was similar in both studies. Any differences in study 2 are highlighted under study 2.

Study 1

Incubators

The Poultry Unit, University of Sydney has 2 identical incubators (Bellsouth Aussieset Incubator, Bellsouth Pty Limited, Narre Warren, Victoria 3805). In study 1, one incubator was set to comply with "ideal" conditions and the other to follow the putative conditions associated with the chick bone weakness syndrome as recorded in a recent field case. The incubation profiles intended are shown in Table 1.

Fertile Eggs

Fertile eggs for study 1 were obtained from a Grand Parent breeding operation. This operation had identified one line of birds as possibly more susceptible to Spraddle Legs at hatch. This operation provided 2400 fertile eggs from the fast feathering line which produces the female breeder at grandparent level for the Cobb broiler (Line 12). Half of these eggs had 5 days storage time and the other half had 10 days storage time when the eggs were placed in the incubators. Ten trays of up to 120 eggs from this donor flock and were sett in each machine, with the storage ages spread evenly between them. After the setter incubation period (days 0 through 18), all the eggs were placed in a single hatcher incubator for hatching on day 22. Machine settings were recorded daily during incubation and embryo temperature was measured on a number days using an infrared temperature detector (Exergen® DX501

Precision IR Thermometer) placed in contact with the shells of 12 randomly selected eggs in each machine. Four data-loggers (AZ 8829 T.RH% Data Logger, Bacto Laboratories Pty Limited, Liverpool, NSW, 2170) were placed strategically in each machine to record actual comparative localised conditions of temperature and humidity. Eggs were weighed in groups prior to incubation and again at 18 days of incubation to determine moisture loss percentages.

At Hatching

At hatch 45 chicks were randomly selected from each incubator, they were blood sampled (serum calcium, phosphorus and magnesium), euthanized and their femurs collected for bone ash determination. Clinically affected chicks from both machines were identified, euthanized and similarly tested. The remaining birds were placed in 8 floor pens according to their incubator and egg storage age groups at a density of approximately 16 birds per m^2 and grown out on commercial broiler rations to 6 weeks of age.

During grow out

Birds demonstrating bone deformity during grow out were recorded, euthanized and necropsied. Forty-four randomly selected birds from each incubator were sampled at 14 days of age for growth plate width (using a CE Electronic Digital calliper), serum Ca, P and Mg and bone ash. At 28 days of age, 44 randomly selected birds per incubator group were sampled for TD score (Figure 3) and serum Calcium, Phosphate and Magnesium. At 42 days of age, 10 randomly selected birds from each pen (40 per incubator group) were examined for locomotory ability using the "Latency-to-Lie" test as described by Weeks *et al*. (2002). In this technique, birds are placed in a tub containing about 3cm of tepid water (approximately 32^{o}C) and observed for the time which the bird takes to attempt to sit down. A maximum time of 5 minutes was allowed for each bird. As soon as each bird made an attempt to sit, it was removed from the tub.

While the birds in study 1 were growing, the incubator trial was repeated (study 2), swapping the incubation regimes to the alternate machine after repairs and service of the malfunctioning incubator were made.

Table 1. Intended incubation profiles

Day of Incubation	Control incubator targets		Test incubator targets	
	Temperature ^{o}F	Relative Humidity %	Temperature ^{o}F	Relative Humidity %
1-2	100.1	58	100.1	55
3-4	100.0	58	100.0	55
5	99.9	58	99.8	55
6	99.8	58	**98.0**	55
7	99.7	58	**99.0**	55
8	99.7	58	**99.0**	55
9-11	98.8	58	99.0	55
12-13	98.6	58	98.6	55
14-18	98.2	58	98.2	55

Major differences in bold type

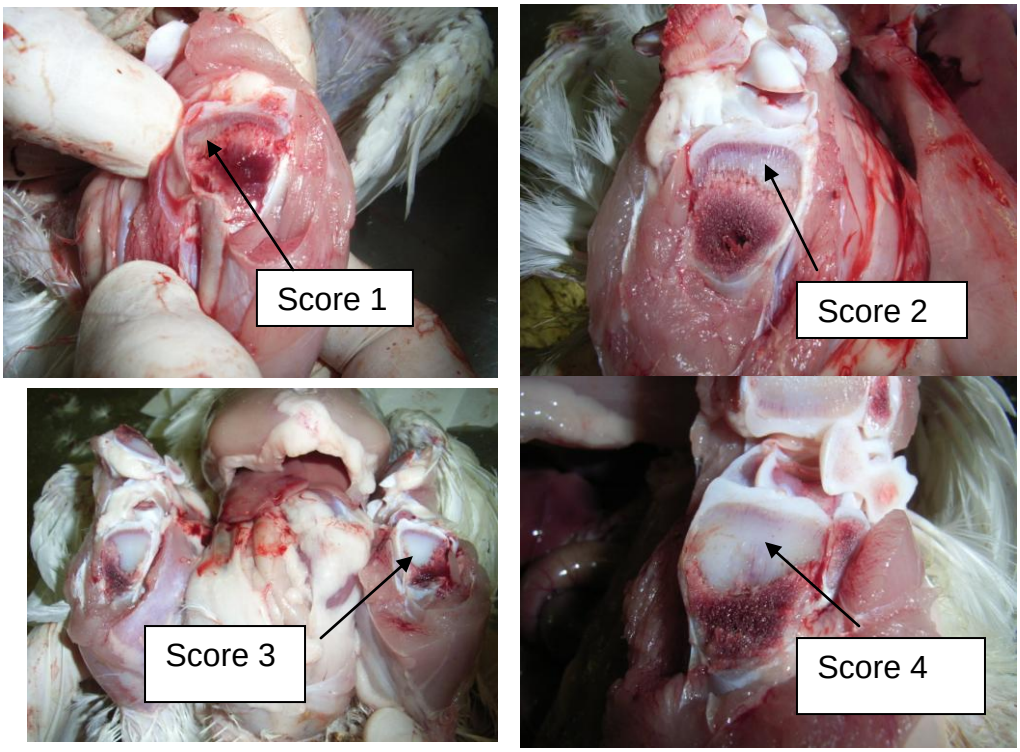

Figure 3. Tibial Dyschondroplasia scores 1-4

Study 2

Based on results from Study 1, the number of eggs required for the repeat study was reduced to 500 (assuming equivalent results would be achieved for the required statistical analysis). Cobb broiler eggs were obtained from a commercial hatchery and randomly assigned to each of the two incubators. The alternate machine that was used in Study 1was assigned to each intended incubation profile (after servicing and repair of the malfunctioning machine). The same intended incubation profile was followed as for Study 1. When hatched, an attempt was made to identify any chicks showing clinical signs of spraddle leg. At hatch, 10 randomly selected chicks from each of the 4 trays of eggs from each group were weighed and measured for beak to toe length (Hill, 2006), bled for serum Ca, P and Mg, humanely euthanized and their femurs were collected for bone ash analysis. Due to the time limitation of this project, the birds from this study could not be grown out and all remaining chicks were euthanized.

Results

Study 1

There were no significant differences in any parameters associated with egg storage times (5 or 10 days in this case) except for hatchability, and no meaningful interactions involving egg storage time and any other measure throughout this study, hence egg age was ignored for the purposes of this analysis.

Table 2 shows the actual incubator settings recorded from each machine for the duration of incubation for study 1. Data-loggers placed in each incubator recorded temperature and relative humidity at hourly intervals. Results on each data-logger in each machine were similar so only records from one recorded in each machine are shown. Comparative results in each incubator are shown in Figures 4 (Temperature) and 5 (Relative Humidity). The

incubator using the Test program functioned very close to the intended pattern. However, from about day 7, the humidity control in the Control incubator malfunctioned, sending relative humidity out of control in this machine. Attempts were made to adjust and fix this but the outcomes were not successful. The results can be seen in the data-logger records. The data-loggers recorded lower temperatures as well as wildly varying humidity. This may reflect different local conditions around the eggs compared with that being recorded by the incubator temperature probe. The intended temperature variation in the test incubator over days 6-8 can be seen to have been achieved in figure 4. Early temperatures in the control incubator were close to intended, certainly during the period where the test incubator profile was varied.

Table 2. Actual Incubator profiles achieved (machine probe and infrared thermometer readings)

Day of Incubation	Control incubator			Test incubator		
	Machine Temp. ^{o}F	Relative Humidity %	Egg shell temperature* ^{o}F	Machine Temp. ^{o}F	Relative Humidity %	Egg shell temperature* ^{o}F
1	100.1	58.1		100.3	55	
2	100.2	58.2	99.2	100.3	55.2	100.7
3	100.1	59.2	99.6	100.1	55	100.0
4	100.2	69.1	99.9	100.1	55.2	100.4
5	100.0	57.1		100.0	55.1	
6	99.9	56.9	100.3	99.8	55	98.7
7	99.7	60	99.1	98.0	55	98.9
8	99.7	63.5	99.0	99.1	55	100.4
9	99.7	Varying	99.1	99.1	55	100.5
10	98.8	64	98.0	99.0	55	100.7
11	98.8	68	98.2	98.9	55	99.8
12	98.8	59.5	98.9	98.9	55	99.9
13	98.6	58.8	97.7	98.6	55	99.6
14	98.5	60.3	98.4	98.6	55	100.8
15	98.2	59.7	98.6	98.3	55	100.0
16	98.2	55	99.1	98.2	55	99.6
17	98.2	52	98.5	98.3	55	99.6
18	98.2	52		98.2	55	

*measured by infrared sensor placed on 10 random eggs per machine

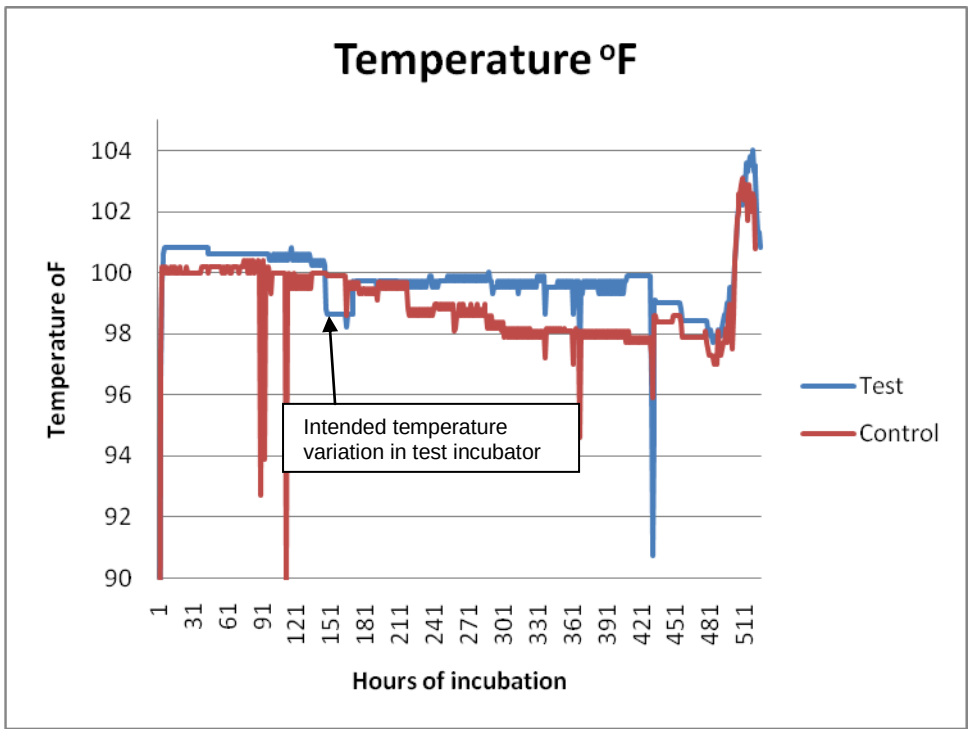

Figure 4. **Data Logger records Study #1 - Temperatures**
(Large drops in records indicate door opened for measurements)

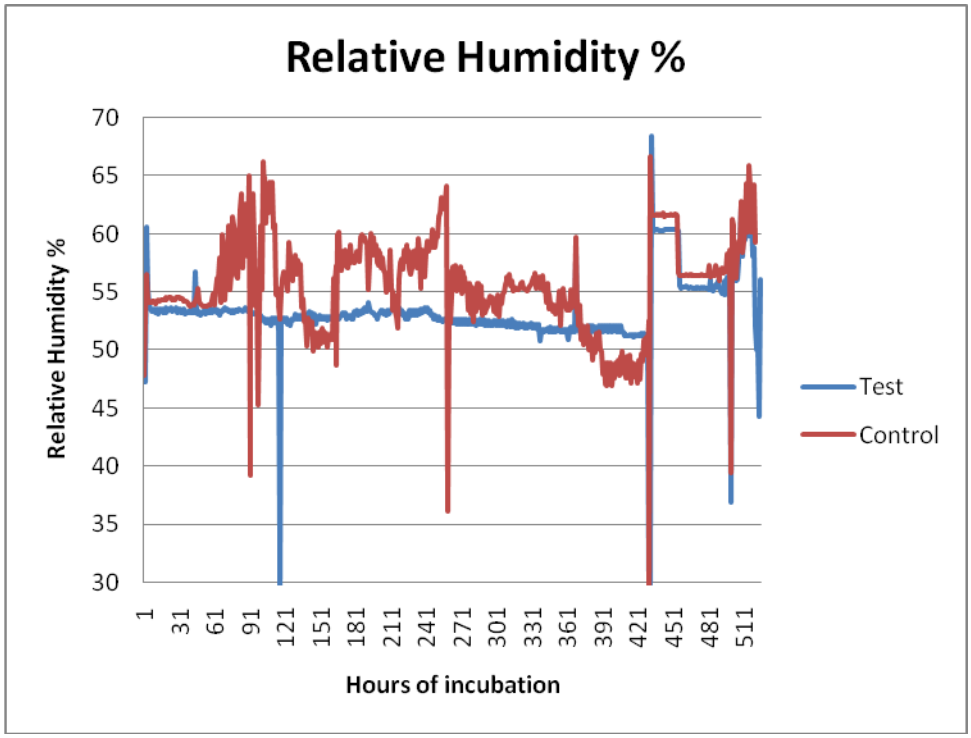

Figure 5. **Data Logger records Study #1 – Relative Humidity**
(Large drops in records indicate door opened for measurements)

As can be seen from the data logger records, machine temperature was also lower than intended from about day 8 and this was reflected by the lower egg shell temperatures observed after day 11.

Table 3. Incubation and hatchability data, study 1

Measurement	Control Incubator		Test Incubator		Probability difference due to chance P=
	Mean	95% Confidence Interval	Mean	95% Confidence Interval	
% Loss in Egg Weight to day 18	**9.44**	9.36 – 9.52	**10.27**	10.26 - 10.28	<0.00001
Total Hatchability %					
5 day egg age	**72.18**	67.04 – 77.32	**77.92**	72.77 – 83.06	0.03
10 day egg age	**66.39**	61.24 – 71.53	**72.33**	67.19 – 77.48	
Cull chicks %*	**1.46**	0.87 – 2.06	**1.55**	0.92 – 2.17	0.83
Spraddle leg chicks %[+]	**1.66**	0.88 – 2.44	**1.83**	1.04 – 2.62	0.75

*As a percentage of eggs sett
[+]As a percentage of total chicks hatched

The malfunction in the control incubator lead to a significant decrease in egg weight loss compared with the test incubator and this was reflected in a lower total hatch percentage for this machine. Higher egg storage age also decreased total hatch in both machines by 5-6%. The reduced egg weight loss also slowed the hatch time for the control machine and chicks came off very wet. Because of this, it was not possible to accurately identify culls from this incubator. An attempt was made to identify obvious splayed leg chicks and these were removed, euthanized and sampled as per methodology from both incubators. The chicks from the control incubator hatched very late, due to the temperature and humidity problems, and were quite wet at take off. This made identification of weak chicks difficult. There was no difference in cull percentage or in percent chicks showing obvious splayed legs at hatch from eggs from either incubator. Bone ash % and serum electrolyte levels of the clinically splay legged birds compared with randomly selected birds are shown in Table 4. In this case, "splay leg" chicks showed a significantly higher bone ash %, lower serum Ca and excessively higher serum P than did the randomly selected group.

Table 4. Differences between randomly selected and clinically splayed leg chicks at hatch.

Chick type	No. chicks	Femur sample weight (mg)	Bone Ash%	Serum Ca (mmol/l)	Serum P (mmol/l)	Serum Mg (mmol/l)
Random	120	0.43^{A}	25.3^{B}	2.09^{A}	1.06^{b}	0.95
Splay legs	36	0.34^{B}	28.7^{A}	1.79^{B}	6.48^{a}	0.94

A,B, means with different superscripts within the same age grouping differ significantly (P<0.01)

Bone ash % results for birds randomly sampled at hatch (day 0), day 14 and day 28 are shown in Table 5.

Table 5. Femoral Bone Ash % and serum electrolyte results at hatch.

Age (days)	Incubator	Bone Ash %	Serum Calcium (mmol/L)	Serum Phosphate (mmol/L)	Serum Magnesium (mmol/L)
0	Test	24.44 [b]	2.20 [A]	1.12 [A]	0.94
	Control	26.26 [a]	1.97 [B]	1.00 [B]	0.96
14	Test	42.97 [b]	1.75	2.01	1.06
	Control	44.08 [a]	1.69	2.03	1.09
28	Test	42.67	2.02	2.05	0.83
	Control	42.84	2.11	2.10	0.84

a,b means with different superscripts within the same age grouping differ significantly (P<0.05)
A,B, means with different superscripts within the same age grouping differ significantly (P<0.01)

Tibial growth plate width analysis and bone ash results at 14 days are shown in Tables 6 and 7. There were no significant correlations between bird weight and growth plate width (r=0.20) or bird weight and bone ash % (r= -.06). Sex however had an effect on growth plate width, but not on bone ash % at 14 days.

Table 6. Tibial Growth Plate Width, day 14

Incubator	Sex	No. birds	Mean Tibial growth plate width (mm)	Standard error of the mean	P=
Control	Female	19	1.97 [B]	0.096	0.0004
	Male	25	2.10 [B]	0.084	
Test	Female	21	2.22 [AB]	0.091	
	Male	23	2.52 [A]	0.087	
Control		44	2.05 [B]	0.353	0.0005
Test		44	2.38 [A]	0.494	
	Female	40	2.10[b]	0.428	0.02
	Male	48	2.30[a]	0.466	

[A,B] Means with different superscripts within a section differ significantly, P<0.001
[a,b] Means with different superscripts within a section differ significantly, P<0.05

Table 7. Femoral bone ash %, day 14

Incubator	Sex	No. birds	Mean Femoral Bone Ash (%)	Standard error of the mean	P=
Control	Female	19	44.4	0.41	0.91
	Male	25	43.8	0.36	
Test	Female	21	43.2	0.39	
	Male	23	42.8	0.37	
Control		44	44.1[A]	0.27	0.004
Test		44	43.0[B]	0.27	
	Female	40	43.8	0.28	0.21
	Male	48	43.3	0.26	

[A,B] Means with different superscripts within a section differ significantly, P<0.01

On day 14 bone ash was significantly lower in chicks from the test incubator (P=0.05), and concurrently these chicks had wider mean tibial plate width (P=0.0005).

Table 8. Growth Plate Width correlation coefficients (r) with Bone Ash at 14 days

Variable	All Birds		Males		Females	
	Ash %	Growth plate mm	Ash %	Growth plate mm	Ash %	Growth plate mm
Ash %	1.00	-0.29*	1.00	-0.42*	1.00	-0.13
Growth plate mm		1.00		1.00		1.00

* r value significant at P<0.05

There was a weak but significant negative correlation between bone ash % and tibial growth plate width at 14 days but this association strengthened to a moderate correlation when only males were considered (Table 8; regression of male bone ash % and tibial growth plate width is shown in figure 5).

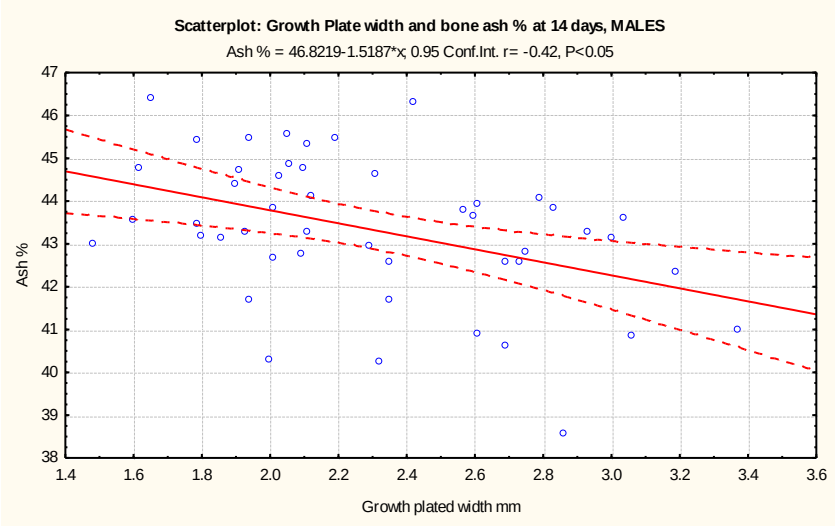

Figure 5. Regression of bone ash % and tibial growth plate width at 14 days in male chickens

There was also a weak but significant negative correlation between tibial growth plate width and serum magnesium (r= -0.26, P<0.05) at 14 days of age.

Table 9 shows relationships between incubator profile and the prevalence of tibial dyschondroplasia (TD) lesions at 28 days of age.

Table 9. Tibial Dyschondroplasia scores at 28 days.

Incubator	No. birds with TD Score						
	0	1	2	3	4	Total TD	% TD
Test	32	5	1	5	1	12	27.3
Control	29	9	5	1	0	15	34.1
Total	61	14	6	6	1	27	30.7
Sex							
Female	32	7	1	0	0	8	20.0 [b]
Male	29	7	5	6	1	19	39.6 [a]

a,b means in the same group with different superscripts differ significantly (P<0.05)

Overall prevalence of TD lesions was 30.7%. There was no significant difference between the occurrences of TD lesions in either incubation group. We observed approximately double

the prevalence of TD lesions in males compared to females however. We could not detect any significant correlations of the prevalence of TD lesions with any serum electrolyte measure at 28 days. Males however had a significantly lower mean serum calcium level than females at this age (P = 0.019). There were no differences between any groups in serum phosphate or magnesium at 28 days.

Table 10 shows the outcome of a Latency-to-Lie (LTL) test carried out on 20 male and 20 female randomly selected birds per group on day 42. Mean live weight of the selected birds are also shown. Although these birds were selected randomly and within the factorial analysis there were only significant differences in weight between males and females, the main effect mean for live weight of the selected birds from the test incubator was heavier than those from the control incubation group by 4% (105gm, P = 0.02). As there was a tendency for heavier birds to have a shorter LTL, this could have inadvertently biased to the comparison, although the effect would have been minimal and there was no significant correlation between weight and LTL time. The LTL data was not normally distributed and was compared using the non-parametric Mann-Whitney U test (Table 10) and by Survival Analysis (Figure 6).

Table 10. Latency to Lie tests at 42 days

Incubator	Egg Age (days)	Sex	Latency time to lie (seconds)	Mean Liveweight (gm)
Test	5	Female	97.0 ^{ab}	2393 ^{B}
		Male	86.8 ^{b}	2887 ^{A}
	10	Female	188.9 ^{ab}	2509 ^{B}
		Male	90.6 ^{b}	2964 ^{A}
Control	5	Female	214.4 ^{a}	2416 ^{B}
		Male	122.8 ^{ab}	2840 ^{A}
	10	Female	192.6 ^{ab}	2355 ^{B}
		Male	137.8 ^{ab}	2721 ^{A}
Main Effects				
Test			115.8 ^{Y}	2688 ^{a}
Control			166.9 ^{X}	2583 ^{b}
	5		130.3	2634
	10		152.5	2638
		Female	173.2 ^{A}	2419 ^{B}
		Male	109.5 ^{B}	2853 ^{A}

A, B means with different superscripts differ significantly (P<0.01) ANOVA
a,b means with different superscripts differ significantly (P<0.05) ANOVA
X,Y means with different superscripts differ significantly (P<0.05) Mann-Whitney U test

Males had a highly significantly shorter LTL time than females. Birds from the test incubator had significantly shorter LTL times than those from the control incubator.

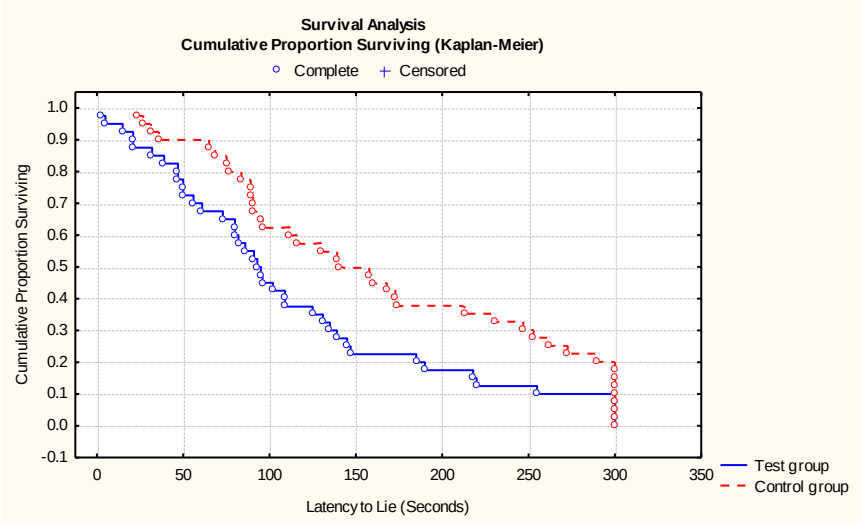

Figure 6. Survival Analysis for Latency To Lie for each incubation group

Survival analysis curves (Figure 6) show the proportion of birds "failing" over time – the graph line shows the number of birds "surviving" (i.e. still standing in this case) at each time point.
The survival curves for LTL times between each incubation group differ significantly (Gehan's Wilcoxon Test, P = 0.02), with the test group showing shorter survival times and fewer birds standing for the full 5 minute test time.

Average sample weights of 30 birds per pen at weekly intervals revealed no significant differences between incubator groups at any age when compared using Repeated Measure ANOVA analysis (see Appendix Figure A1).

Overall losses of birds due to mortality or culling are shown in Table 11.

Table 11. Causes of bird losses in Study 1

Incubation group	Number hatched	Weak chicks in first week	Femoral Head Necrosis	Rotated Tibia	Varus-Valgus deformity	Ascites	Other*
Control	909	11	10	1	6	6	14
Test	932	5	4	3	1	9	17
P value from χ^2 analysis		*0.12*	*0.10*	*0.33*	*0.07*	*0.47*	*0.64*

*includes SDS, colibacillosis, heat stress

The malfunctioning incubation conditions in the Control group may have contributed to the high early losses and possibly exacerbated birds susceptible to femoral head necrosis and varus-valgus deformity. There was no increase in clinical skeletal problems observed in the Test incubation group.

Study 2

Records of the incubator readouts for along with egg shell temperature recordings from study 2 are shown in table 12. It became clear that the incubator being used for the test group was not actually achieving the temperatures claimed by the machine readout and was running below the set points. Adjustments were attempted but the conditions were not able to be corrected during the study.

Table 12. Incubator probe readings and Egg Shell temperature records for Study 2.

Day of Incubation	Control incubator			Test incubator		
	Machine Temp. °F	Relative Humidity %	Egg shell temperature* °F	Machine Temp. °F	Relative Humidity %	Egg shell temperature* °F
1	100.1	58		100.1	54	
2	100.1	58	100.6	100.1	54.9	96.4
3	100.1	58	100.9	100.1	54.9	98.3
4	100.1	58	101.3	100	54.9	98.0
5	99.9	58	100.9	100	54.9	97.4
6	99.8	58	100.9	99.8	54.9	94.8
7	99.6	58		98.1	55	
8	99.6	58	100.7	98.9	54.9	95.8
9	99.7	58		99	54	
10	99.8	58		99.1	55	
11	98.7	58	99.8	99.1	55.2	97.5
12	98.7	58	100.5	99.1	54.7	97.2
13	98.7	58		98.7	54.7	
14	98.8	58	100.4	98.3	>100	98.9
15	98.7	58		98.4	54.9	
16	98.3	58.1	100.7	98.2	54.6	99.1
17	98.3	58	101.0	98.1	54.7	99.3
18	98.4	58		98.2	54.9	

A decrease in the temperature was achieved at day 6 but this was more extreme than intended and the subsequent planned rise in temperature on day 7 was not achieved. The test machine ran cool for most of its incubation time. A summary of the data logger records from these two machines are shown in figures 7 and 8, showing the poor performance of the test machine. As a result the hatch from this machine was very slow, with many chicks failing to hatch at the required time. This limited the number of chicks for sampling at hatch.

The data loggers placed in each machine showed marked variation from the intended profile in the Test group incubator in study 2. Actual air temperatures achieved in the test incubator were extremely low, reflecting the egg shell temperatures observed (Table 12).

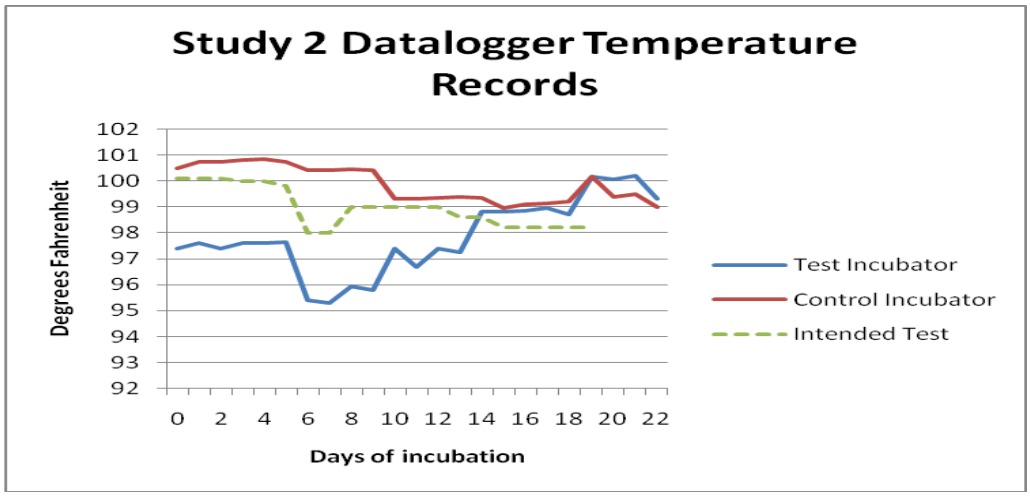

Figure 7. Datalogger Temperature records from each machine (compared with the intended test profile), Study 2.

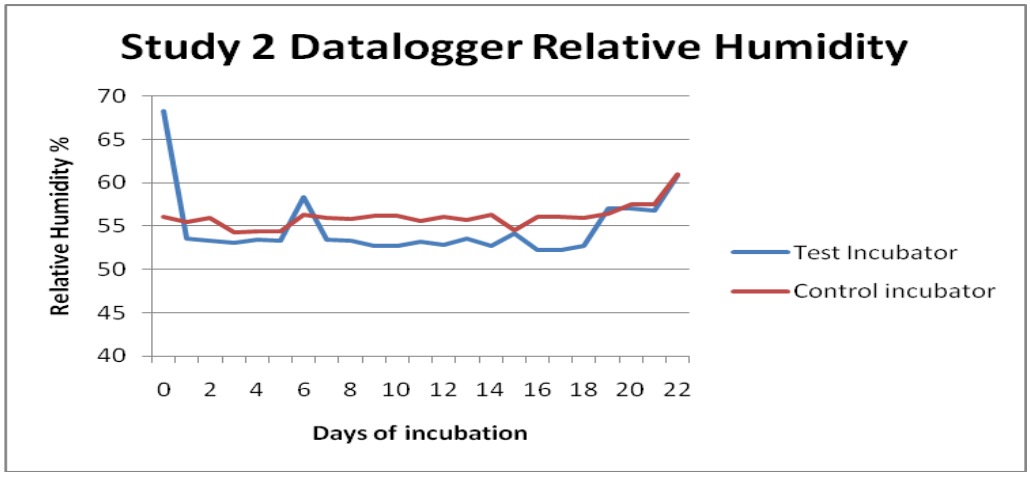

Figure 8. Datalogger Relative Humidity records for each machine, Study 2

Table 13. Measurements from chicks hatched in Study 2.

Incubation group	No. chicks measured	Chick Weight (gm)	Chick length (cm)	Bone sample weight (gm)	Bone Ash %
Control	46	42.2	18.7	0.50	27.0
Test	37	42.3	17.4	0.36	30.9
	P=	*0.96*	*<0.0001*	*0.003*	*<0.0001*

While chick weight between the groups did not differ, there were major differences in chick length, weight of femurs used for the bone ash sample and bone ash % between the incubation groups at hatch. Bone ash % and sample weight showed a reasonably strong negative correlation (r= -0.58) and the association appeared to better fit a polynomial relationship (Figure 9).

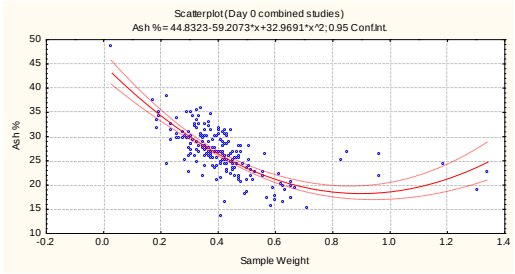

Figure 9. Regression of Bone Ash % on Weight of the bone sample used, studies 1 & 2 combined

Comparisons of bone ash results from both studies were conducted (Table 14). The bone ash % for the control incubation groups were similar while differing from the Test group in study 1 and all groups differed significantly from the test group in study 2.

Table 14. Bone Ash % comparisons over both incubation studies

Study No.	Incubation treatment	No. Chicks	Bone Ash %
1	Control	60	26.3^{b}
	Test	60	24.4^{c}
2	Control	46	27.0^{b}
	Test	37	30.9^{a}

a,b,c – means with different superscripts differ significantly (P<0.05)

Discussion

The background information leading to the incubation temperature and humidity differences used in this study originated from a commercial hatchery that experienced two hatching occurrences that resulted in an unusually high prevalence of splay legs and weak boned chicks. This resulted in high cull numbers in the first week of their lives. Some records of machine temperatures and humidity were recorded by the hatchery and these were compared to records from contemporaneous hatches of eggs from the same breeder flock where this problem was not observed. The major detectable difference involved a temperature drop of almost 2^{o}F on day 5-6 of incubation followed by an increase back to the desired level on day 7. There was also a slightly lower relative humidity in one of the problem groups. The profile selected for study (Table 1) was based on these two occurrences.

Despite the malfunctioning of the control incubator in Study 1, we were able to demonstrate differences in skeletal integrity due to a change in early incubation conditions. This involved a decrease in set temperature on day 6 followed by a temperature rise on day 7 of incubation. While this sort of whole machine change would be uncommon in commercial hatcheries, the existence of hot or cold spots in incubators which may vary under particular ventilation changes may create local conditions that could affect considerable numbers of eggs. This in part may explain the variable expression of the described clinical condition in newly hatched chicks in the field.

Randomly selected chicks incubated under the test conditions had significantly lower femoral bone ash % and higher serum calcium and phosphate than those from the control incubator. Bone and serum calcium levels are under the control of two hormones; calcitonin, secreted by the thyroid gland, and parathyroid hormone (PTH - from the parathyroid glands). Calcitonin acts against increased serum Ca levels and promotes Ca movement into bone tissue (Bowen, 2006). PTH has a powerful effect on bone cells and causes them to release Ca into the blood stream (Cunningham, 1992). It is possible the observed patterns may relate to an imbalance of one or both of these hormones. Pines (2007) hypothesised that temperature variation effects in the first 8 days of incubation could adversely impact the thyroid and parathyroid glands which are undergoing development during that period. The higher serum electrolyte levels reveal that this condition is obviously not of simple aetiology and remains to be explained more fully.

Growing birds from each incubation group were shown to differ significantly in femoral bone ash % and tibial growth plate width at 14 days. A widening of the epiphyseal growth plate is possibly consistent with sub-clinical rickets (Thorp, 2008), visibly similar to that described under conditions of dietary phosphorus deficiency (Klasing, 2008). This was more apparent in male birds and there was a moderately strong significant negative correlation between tibial growth plate width and bone ash % at this age, mainly in male birds.

The traditional method of assessing locomotory capability of broiler chickens has been to estimate a "gait score" in older broilers (Kestin *et al*., 1992). This involves observing birds walking over a distance of at least 2 m within a shed environment. This is difficult to estimate in small pens as the birds do not have long straight run areas and there is much furniture blocking their path. The "Latency To Lie" technique was developed as a more objective test of locomotory ability and has been shown to correlate well with gait score methodology (Weeks *et al*., 2008). Haslam (2008) has also suggested that this is a more welfare friendly approach to assessing a bird's leg strength and that birds which quickly overcome their aversion to sit in water are experiencing some degree of pain.

The grow out of the birds in Study 1 did not exhibit varying levels of clinical leg problems between the incubation groups but measurable differences in mobility was demonstrated at 42 days by "Latency-to-Lie" (LTL) testing. It is thought that many management conditions can affect the incidence of leg problems seen in the field and a strong focus has been placed on stocking density in this respect (Knowles et al., 2008; Haslam, 2008). The stocking density used in this experiment was much lighter than used in the commercial field in similar shedding types (16 birds/m^2 here compared to 18-20 birds/m^2 in the field) and this may have decreased opportunities to display these signs under our conditions. However, LTL testing was able to demonstrate marked differences between the incubation programs. This needs to be considered in the light of the slightly higher live weight of the randomly selected birds from the test incubation group in this study, but the difference in weight was marginal (4%) and the correlation between weight and LTL time was low (r = -0.28).

There is little documented information on spraddle legs. Crespo and Shivaprasad (2008) suggested that a higher prevalence of spraddle legs occurred in association with higher humidity during incubation. The control incubator in Study 1 produced markedly elevated humidity throughout most of the incubation accompanied by grossly inadequate egg weight loss, but this did not show an association with poorer bone ash levels. So our findings here may conflict with the published observations. The Control group however did experience marginally more femoral head necrosis and varus-valgus deformity than the test group and it may be worth pursuing this observation in future studies.
Genin *et al.* (2008) implied that a cyclic temperature variation in early incubation (rises in temperature for 6 hours over each of days 1-8) was associated with a higher prevalence of tibial dyschondroplasia. The present study utilised a temperature drop of 2^oF on day 6 followed by an increase back to the control temperature by day 8, and thus varied from the profile used by those authors. The conditions used in the present study did not induce an increase in TD lesions in comparison to the control incubation.

Results from study 2 were complicated by the continued malfunctioning of the incubator used for the Test group. The actual incubation profile achieved did not resemble the intended test regime at all. Chick length is a measure of the efficiency of the incubation process (Hill, 2006) and is not correlated to chick weight. Chick length is an indication of utilisation of the yolk during incubation – longer chicks will have utilised more yolk and are considered of better viability and likely to have better performance in the field. The much shorter chicks hatched from the test incubator under these poor conditions had much higher bone ash % than the control group. Control groups in both studies did not differ in bone ash %. Shorter chicks would have necessarily had shorter bones and we can see a negative correlation between sample weight (a function of the bone size) and bone ash % (Figure 9). This may reflect a difference in mineral density related to bone surface area: volume ratio, cross sectional area or width. The observed difference in bone ash here may not represent bone quality in this case as the bones on these birds were observed to be soft. Chick length and weight were not measured in study 1, but sampled bone size for clinically splay legged chicks were lower than for the randomly selected birds and a similar distortion of the results may have occurred in this situation. It would seem that bone ash % at hatch may be a complicated parameter to assess without other collaborative measurements and interpretation of these results in clinically affected chicks may be complex.

Implications

Recent published findings and the outcomes of this study show strong indications that incubation conditions can affect bone characteristics at hatch and the incidence of later skeletal abnormalities and mobility during broiler growth. As leg weaknesses represent a major welfare concern in modern broiler chickens it is important that these incubation associations are better defined so that measures can be taken by hatcheries to minimise the potential problems that may eventuate in the field. With the current growth rate of modern broiler breeds and a life expectancy of 6-7 weeks, the incubation period represents at least 30% of a broiler's total commercial lifespan and is thus has significant impact on its overall growth, health and performance.

Recommendations

1. It appears as a priority that the incidence of these weak bone conditions which may or may not be obvious at hatching be estimated in commercial broiler hatcheries and farms under Australian conditions. This would require an epidemiological survey of a number of hatcheries over a reasonable period, attempting to assess the occurrence of skeletal conditions between individual setter incubators and obtaining whatever historic incubation condition records exist for these machines. This will be much more difficult in the field where birds from several incubators are combined in growing sheds. Statistical associations could then be sought to elucidate putative conditions which may be associated with incidence. Purpose designed surveys could utilise recording devices placed in selected incubators and assessing chicks from these machines at hatch and grown out under controlled conditions. If possible, broiler batches from these hatches could be followed through in the field for incidence of leg abnormalities

2. The underlying embryological and physiological mechanisms underlying the development of these problems during incubation need to better understood. This would require reliable, small scale experimental incubators with advanced recording capabilities. Embryonic bone growth is not covered well by the scientific literature, especially not in regard to modern broiler breeds, which have changed so much in metabolism due to genetic selection for growth and muscle mass. The basic underlying bone metabolism may also have been modified.

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Appendix

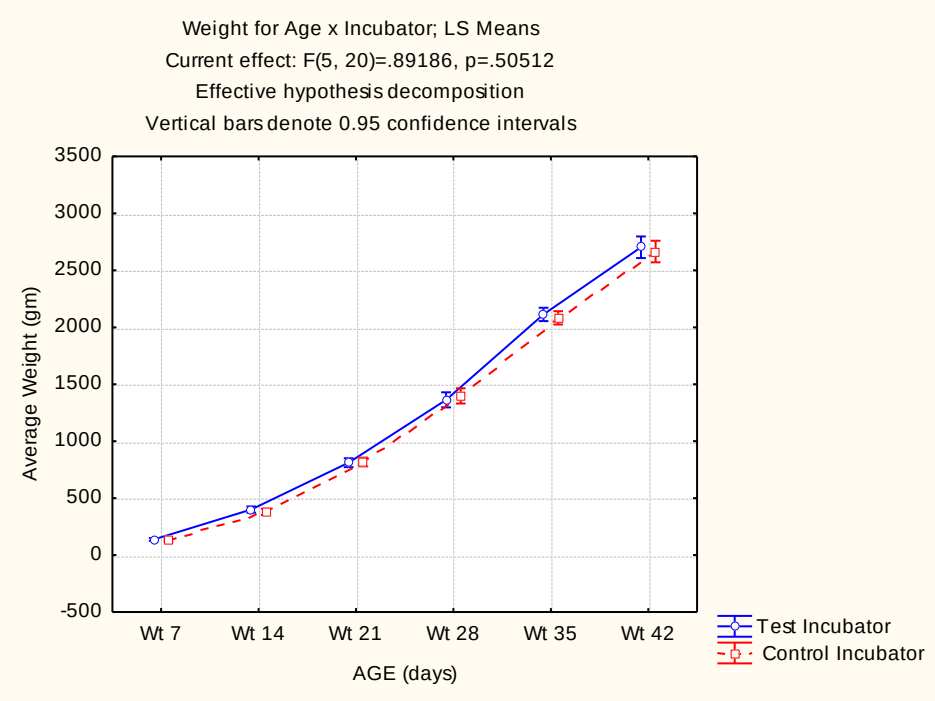

Figure A1. Growth patterns of birds in Study 1

Glossary

Bone Ash	analysis of the degree of bone mineralisation where the bone is heated at 600ºC until there is no further weight loss
Rotated Tibia	Lateral rotation of the distal tibia on its long axis
Spraddle or Splay Leg	Lateral deviation of the leg
Tibial Dyschondroplasia	Growth plate cartilage abnormality – failure of removal of avascular chondrocytes
Tibial Growth Plate	The epiphyseal plate – cartilaginous region at the proximal end of the tibia and is the site of growth in length of the bone
Valgus	"Bent outward" – angulation away from the midline
Varus	"Bent inward" – angulation towards the mid line
Varus-valgus deformity	Also called "perosis" which involves subluxation of the gastrocnemius tendon, which is secondary to long bone shortening caused by damage to the growth plate (chondrodystrophy).

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Plain English Compendium Summary

Project Title:	Broiler chicken skeletal integrity and incubation
Project No.:	09-24
Researcher:	Peter Groves
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Objectives	
Background	Leg weakness is a major welfare concern in modern broiler chickens. There are several described conditions which contribute to these leg problems. Observations have implicated a possible role for variations in incubation conditions (temperature and humidity) in the development of some of these conditions.
Research	The research attempted to replicate an incubation profile which may have been associated with an increased level of leg problems in a flock. There were only minor differences from "ideal" conditions used in this profile.
Outcomes	Differences in bone ash %, serum calcium and phosphate levels, growth plate width at 14 days and an estimate of the birds leg strength were discovered associated with the modified incubation profile over a 6 week growth period.
Implications	An overall assessment of the impact of variations in incubation conditions which may occur in the field need to be studied and an understanding of the impact of minor incubation variations need to be gained. Improved knowledge here may allow for improvements in bird welfare in commercial situations.
Publications	