Effects of breeder nutrition and management, and incubation on broiler leg health

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Summary

The majority of interventions to reduce the prevalence of poultry leg issues have been done at farm level. Nevertheless, developmental disorders of the poultry locomotion system could start during embryo development. Several locomotion problems are observed at hatch or days later at the farms; however, very little research has been done or attention been placed on factors at hatchery and breeder levels where these problems may initiate. Breeder nutrition and management play key roles on egg traits that may affect progeny embryo development. The objective of this presentation is to discuss some nutritional and management factors for the breeders, and incubation conditions that can affect early development of the whole locomotion system and the voluntary physical activity of broilers. Possible negative effects are finally observed on increased prevalence of leg problems and reduced bone strength in broiler flocks.

Key words: leg problems, locomotion, breeders, incubation, management, nutrition.

Importance of leg problems in broiler chickens

Leg problems are one of the most common causes of culling, early and late mortality in broiler flocks, and are considered to be one of the more serious welfare problems facing the broiler industry (SCAHAW, 2000; Mench, 2004). In broiler flocks worldwide it is common to observe chickens with some type of leg problem, and because of this it is important to determine the prevalence and not the incidence of these abnormalities. The median prevalence of observable problems is between 1 and 3%. Chickens with locomotion problems can be easily identified at hatch or during the first days after placement in the poultry houses with splayed or twisted legs or paralysis (Oviedo-Rondón and Wineland, 2011). In the following weeks, severe cases of valgus, varus, crooked toes, general lameness or paralysis are common causes of elimination due to welfare concerns. Broiler chickens that are not severely affected by these disorders can constitute at least 20 to 30% of the flock and can show different degrees of modification in their normal gait. The prevalence values of locomotion issues change during the year and according to the region in the world (Kestin *et al*., 1992; Sanotra *et al*., 2001; Bradshaw *et al*., 2002; Mench, 2004).

The disorders of the chicken locomotion system and bone fragility have an important impact on animal welfare audits, the physical and microbiological quality of carcasses and on the production costs (Sullivan, 1994; SCAHAW, 2000; Mench, 2004). The reduced physical activity of chickens and the abnormal alignment of bones in the tibiotarsal articulation (varus/valgus) have been correlated with higher numbers of scratches, breast blisters and carcass contamination at processing (Vaillancourt and Martínez, 2002). Leg problems are also associated with unevenness of bilateral traits (Møller *et al*., 1999), a biological phenomenon called bilateral asymmetry that can be measured as relative asymmetry (**RA**) as described by Møller and Swaddle (1997). The asymmetry in bilateral carcass parts occasionally causes problems in processing lines and automatic deboning. These asymmetries increase the need for manual intervention, carcass trimming and downgrading. In a similar manner, bone fragility and porosity in tibia and femur epiphyses constitute a quality problem in the final product, because they cause darkening of bones. This discoloration extends to the surrounding meat during cooking causing the black bone syndrome. Meat discolorations in totally cooked poultry products can cause up to 11% of rejections, making this syndrome an important cause of rejection, especially in thighs and breast (Smith and Northcutt, 2003).

Another point of view on bone and locomotion issues in broilers

The locomotion system includes the bones, muscles, tendons, cartilage and synovial fluid in the articulations. Additionally the neural system plays a fundamental role in stimulation of muscles for contraction and movement (Muir *et al*., 1996, 1998). Bones are generally the only component evaluated when leg or locomotion problems are observed in poultry flocks or in poultry research due to size and ease of assessment. However, it is important to remember the other components of the locomotion system and that correct alignment of the parts are necessary to obtain adequate locomotion function in bipedal animals (Abourachid, 2000; Bizeray *et al*., 2001; Oviedo-Rondón, 2007). For example, tendon strength and function is critical for avian locomotion (Foutz *et al*., 2007). Bone deformities have been well described (Riddell, 1992; Bradshaw *et al*., 2002, Ito *et al*., 2010). These skeletal disorders are common and easy to observe, but very little is known about tendons, cartilage, synovial fluid, neural innervations, and biomechanics of movement in broilers and other avian species. This is one of the reasons that determining definite solutions to leg problems in poultry has been so difficult in the past 30 years. More research in this area is needed.

Many problems of paralysis are related to neurological problems affecting the spinal cord (Ito *et al*., 2010). In broilers, thoracic vertebrae are articulated, take longer to fuse than in other species, and even after vertebrae fusion the sixth thoracic vertebra articulates with the sinsacrum. Consequently, displacements of these vertebrae are observed causing kyphosis or lordoses with compression of the spinal cord. Currently, other conditions like scoliosis, spondylolisthesis and vertebral abscesses contaminated with bacteria like *Estafilococos*, *E. coli* or *Enterococus cecorum* are more frequently reported (Makrai *et al*., 2011; Armour *et al*., 2011).

These deformations of the vertebral column have been associated in humans with asymmetric development (Stokes *et al*., 2006; Peterson and Wenger, 2008). Embryo developmental asymmetry can cause unevenness in several bilateral characteristics of animals and the spinal loading asymmetry is considered the initial cause of scoliosis (Møller *et al*., 1999; Møller and Manning, 2003; Stokes *et al*., 2006). In broilers some mild spinal injuries can cause modifications in gait, and further they can progress to paralysis or asymmetry during leg bone development (Muir *et al*., 1998). When growth and development of leg bones are not symmetric, the animal adopts different standing positions and gaits to compensate for changes in the center of gravity and to maintain equilibrium during locomotion (Muir *et al*., 1998). Any changes in gait may impose forces on bones which can cause deformation or articulation misalignment. Inadequate position occasionally affects the development of the vertebral column (Stokes *et al*., 2006; Peterson and Wenger, 2008). Droual *et al*., (1991) reported an association between scoliosis and angular changes in tibiotarsial articulation in broilers. Even though this work did not show a direct causal relationship, it suggested that leg deformities can affect the vertebral column, as has been reported in humans.

Management and nutrition in breeders and leg problems in progeny

Nutrition and feeding of broiler breeders are fundamental for adequate development of their progeny (Kidd, 2003; Calini and Sirri, 2007). Embryo development is totally dependent upon egg nutrients deposited by hens. Specific nutrients such us vitamin D, trace minerals, and fatty acids seem to play key roles in early bone development (Kidd, 2003; Oviedo-Rondón *et al*., 2006a; Calini and Sirri, 2007). Hens also affect embryo nutrition and development by eggshell properties such as egg porosity and shell thickness which determines conductance. Eggshell conductance determines the capacity of eggs to exchange gases and water vapor, consequently affecting embryo yolk and general nutrient utilization. These physical factors, especially the capacity to obtain sufficient oxygen, determine the type of metabolism, rates of tissue development, and embryo growth. This is more important during the last three or four days prior to hatch, in the plateau stage of oxygen consumption (Rahn *et al*., 1979) when development of many tissues including bones and muscles is at its fastest rate (Applegate and Lilburn, 2002).

We have observed significant correlations between eggshell conductance and residual yolk at hatch with bone development in several studies **(Table 1)**. These correlations are weak, but indicate that embryos from eggs with higher eggshell conductance have more bone development at hatch. More residual yolk at hatch, indicates reduced uptake of nutrients by the embryo, leading to lower bone development. Eggshell and albumen properties, and yolk components are affected by broiler breeder management and nutrition during rearing and laying phases. Breeder management that cause under nutrition or stress produce negative changes in egg properties important for embryo development (Kidd, 2003; Hocking, 2009).

Table 1 - Correlation coefficients between eggshell conductance, residual yolk and bone traits of the progeny at hatch.

Incubation variables	Progeny bone traits at hatch		r^1	P- value
Eggshell conductance	Relative weights (%)	Femur	0.38	0.005
		Tibia	0.28	0.012
		Metatarsus	0.29	0.025
	Length	Femur	0.53	<0.0001
		Tibia	0.58	<0.001
		Metatarsus	0.60	<0.001
Residual yolk at hatch, %	Relative weights (%)	Femur	-0.48	<0.001
		Tibia	-0.57	<0.001
		Metatarsus	-0.27	0.050
	Length	Femur	-0.37	0.002
		Tibia	-0.39	<0.001
		Metatarsus	-0.37	<0.001

1 - 110 observation were used for these correlations.

Our research group has conducted several experiments (Eusebio-Balcazar *et al.*, 2009 a,b,c; 2010; Oviedo-Rondón *et al.*, 2008a; 2010, 2011) to evaluate the effects of nutrition and breeder management on the incidence of leg problems in broilers. We have evaluated factors such as genetics, cereal type (corn *vs*. wheat), source of trace minerals (inorganics *vs*. organic), breeder age, feed restriction (*ad libitum vs*. feed restricted), feed restriction programs (every day feeding *vs.* skip a day), feeding programs (g/pullet/day) during breeder pullet rearing, and changes in feeder space from the rearing to the production house. The results of these studies indicated that some of these factors may impact egg traits important for embryo development. For example, the last two factors listed affected moisture loss and eggshell conductance **(Table 2)**. In these studies, it was also corroborated that there are differences between commercial genetic lines of broilers in eggshell percentage and thickness of the albumen and eggshell membrane. This suggests that genetic lines differ in eggshell conductance (Hamidu *et al.*, 2007). In broiler genetic lines of low eggshell conductance, heat stress and low oxygen levels in the hatchers can cause major impacts on embryo development.

Differences in the prevalence of leg problems have been reported among genetic lines, but these variations could be due to effects of eggshell properties on embryo development. Increasing feeder space from rearing to production caused breeders to change eggshell conductance **(Table 2)**, affecting progeny bone development at hatch **(Table 3)**, and the probability to observe locomotion issues in the progeny at six weeks of age **(Figure1)**. In our experiments, changes in fertile egg traits due to breeder management have always been correlated with changes in progeny bone development at hatch and leg disorders and locomotion issues in broilers at 4 and 6 weeks of age. Progeny of genetic lines with faster growth and voracity in feed intake had less severe problems of valgus when breeders were fed according to a program with more feed restriction between 14 and 20 weeks of age and when the feeder space offered per breeder hen was similar or lower to the one that these breeders had as pullets during the rearing phase.

In other experiments (Oviedo-Rondón *et al.*, 2008a; Eusebio-Balcazar *et al.*, 2010) the supplementation of organic trace minerals (**OTM**) like zinc, copper, manganese and selenium was evaluated either as additional source of trace minerals on top of the inorganic premix or to partially (30%) replace inorganic sources with chelates in broiler breeder diets. In these studies, eggs were subjected to elevated incubation temperatures in the hatchers. The results of these studies indicated that OTM in breeder diets improved eggshell strength, increased

Table 2 - Effects of cereal type in breeder feed, feeding program between 14 and 29 weeks of age and change in feeder space from rearing to laying phase on egg weight, egg moisture loss, and eggshell conductance in 33 week-old Cobb 500 breeders

Breeder treatments			Egg weight (g)	Moisture loss (%)	Eggshell conductance (mg of H_2O/mmHg)
Cereal[1]	Feeding program (14 – 29 wk)[2]	Change in feeder space[3]			
Corn			59.70	8.91	13.73
Wheat			59.51	8.85	13.53
	Fast		59.81a	8.80b	13.50b
	Slow		59.40b	8.96a	13.75a
		More	59.37b	9.00a	13.78a
		Similar	59.84a	8.76b	13.48b

a-b Means within the same column of similar treatment without common letter are significantly different (P < 0.05). 1 - Breeders and progeny were fed diets based on corn or wheat during all their life. 2 - Feed was offered according to two feeding programs with fast or slow increments in amounts of feed (g/pullet) between 14 and 29 of life. 3 - At photo stimulation (22 weeks), breeders were transferred to a laying house with feeder space similar or higher (6.3-6.5 *vs*. 6.3-8.4 cm/hen) than the one offered to pullets.

Table 3 - Effect of feeder space change from rearing to production[1] on progeny bone traits at hatch in 33-week old Cobb 500 breeders.

Feeder space change[1]	Relative bone weight in relation to weight of chicks without yolk			Length			Thickness of metatarsus
	Tibia	Femur	Metatarsus	Tibia	Femur	Metatarsus	
	----------- (%) -----------			------------------- (mm) -------------------			
More	0.89a	0.53	3.05	30.56a	21.91a	23.95a	3.57b
Similar	0.86b	0.53	3.01	30.24b	21.69b	23.70b	3.68a

a-b Means within the same column of similar treatment without common letter are significantly different (P < 0.05).
1 At photo stimulation (22 weeks), breeders were transferred to a laying house with feeder space similar or higher (6.3-6.5 vs. 6.3-8.4 cm/hen) than the one offered to pullets.

progeny relative bone weight at hatch and reduced RA of metatarsus. Furthermore, it was possible to observe that chicken offspring from breeders fed diets with OTM showed fewer locomotion problems and higher tibia strength at 49 days of age.

Role of Incubation on bone development and locomotion problems

Suboptimal incubation conditions are difficult to completely eliminate in incubators and hatchers under commercial conditions due to the great variability of egg traits and the large capacity of the machines (French, 1997, 1999). Microenvironments with unfavorable conditions for embryo development may occur especially in multistage incubator machines. Adverse incubation temperatures and oxygen levels can affect nutrient absorption from yolk, hormonal control, development of several other tissues (Christensen *et al*., 2002; Wineland *et al*., 2006a, b; Oviedo-Rondón *et al*., 2006b, 2008b, c) and increase developmental asymmetry (Eriksen *et al*., 2003). For example, conjunctive tissue and bone ossification starts during the embryonic phase (Ballock and O'Keefe, 2003) and the highest growth rate of bone occurs just days prior to hatch and a few days post-hatch (Church and Johnson, 1964; Applegate and Lilburn, 2002). This explains why when incubation conditions are stressful enough for embryos, many leg issues are observed at hatching or weeks after (Oviedo-Rondón *et al*., 2008 b, c, 2009a; Oviedo-Rondón and Wineland, 2011).

Small differences in incubation temperatures applied throughout incubation have been previously shown to influence growth of the long bones in the

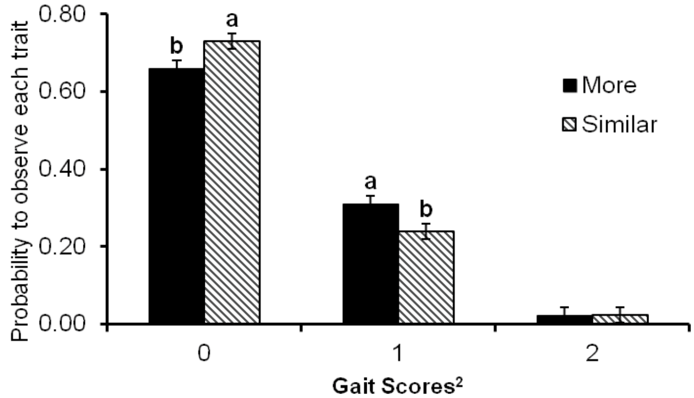

Figure 1 - Average effect of changes in breeder feeder space from rearing to production1 on probability to observe specific gait scores at 42 days in progeny of Cobb 500 breeders at 32 and 44-weeks of age. a-bMeans within the same column of similar treatment without common letter are significantly different (P<0.05). 1 - Breeders at photostimulation (22 weeks) were transferred in a house equipped with feeders to offer similar of more space during the laying phase (6.3-6.5 *vs*. 6.3-8.4 cm/breeder) compared to rearing space. Data correspond to probabilities to observe each gait score calculated from evaluating either 768 or 512 chickens per treatment in two experiments. 2 - The "gait scores" were evaluated according to Webster *et al*., (2008).

chick leg (Brookes and May, 1972). Hammond *et al*., (2007) concluded that raising the temperature of the eggs from 37.5 °C (99.5 °F) to 38.5 °C (101.3 °F), during E4-E7 can increase the length of tibia and tarsus bones. Shim and Pesti (2011) tested the hypothesis that increasing or decreasing the temperature of chick incubation by 1 °C for 3 d during ED 4 to 7 affects hatchability, growth, and leg abnormalities. These authors used Cobb 500 broilers fed 3 diets: a diet that induced tibial dyschondroplasia (TD), a Ca-deficient diet that induced rickets, and a P-deficient diet that induced rickets. Shim and Pesti (2011) did not detect main effects or interactions between incubation temperatures during days 3 to 7, or time and bone abnormalities. The authors emphasized that treatments affected hatching time, but their hatcher temperatures were maintained at only 36.1 °C (97 °F) and 70% RH for the last 54 hours of incubation, which is a lower temperature than normally observed under commercial conditions especially in multistage machines. This indicates that these chickens did not suffer the normal stress of other studies during the last phase of incubation.

Chondrocyte proliferation and differentiation are affected by temperature stress, especially close to hatch. The research group of Yalçin and collaborators (2007) showed how less than or greater than optimal incubation temperatures influenced the incidence of TD at 49 d of age. In this study TD incidence was higher (14.4 and 12.8% vs 5.0%) for chickens exposed to cool (36.9oC; 98.4 °F) or hot (39.6 °C; 103.3oF) temperatures between E0 and E8, compared to chickens exposed to optimum conditions. In this experiment the tibia weight at hatching and at 49 d of age was reduced by heating eggs to 39.6 °C (103.3 °F) for 6 h/day during E10 to E18. The same authors showed the effects of incubation temperatures on several bone proteins. The previous research reports indicated that effects of temperature during incubation on bone development and bone deformities post-hatch depend on the time that stress is applied and severity of this stress.

Our research group has considered that the most critical times for embryo development are the first and last stages of development. These are also periods difficult to manage under commercial conditions. Management of eggs prior to incubation and in the hatchers generally is not optimum in commercial hatcheries due to several factors. We have observed (Oviedo-Rondón *et al*., 2008 a; Oviedo-Rondón, 2009a, b, c) that adequately pre-warming eggs with good air flow and avoiding low temperatures during early incubation are critical for adequate bone development, reduced bone RA between both legs and to minimize incidence of toe deformities and leg bone problems. On the other hand, high incubation temperatures and hypoxia during the last phase of incubation can decrease bone development, increase bone asymmetry between both legs, and down-regulate expression of genes and proteins like collagen type X, growth factor TGF-, metalloproteinases, which are important molecules for adequate ossification (Eusebio-Balcazar *et al*., 2009a, b, c; Oviedo-Rondón *et al*., 2008 b, c; Oviedo-Rondón, 2009a, b).

The beneficial effects of adequate incubation temperature profiles and good ventilation on leg problems have also been observed in chickens under commercial conditions during 6 hatches in flocks of 80,000 broilers each in a commercial incubator and in commercial farms (Oviedo-Rondón *et al*., 2009b). Results **(Table 4)** indicated that chickens obtained in an incubation profile better controlled in single stage machines had lower prevalence of crooked toes and better gait scores (Gait score 0) than chickens coming out of multiple stage machines where temperature conditions are more difficult to manage.

Table 4 - Percentage of broilers within each category of gait score at 56 days of age according to two incubation temperature profiles evaluated in a commercial hatchery and commercial farms (Oviedo-Rondón *et al*, 2009b).

Incubation profile	Sex	Gait scores[1] 0	1	2	3	4	5
		%					
Multi-stage[2]	Males	34.00	50.75	12.25	2.00	0.50	0.00
	Females	72.38	23.63	2.63	0.75	0.00	0.00
	Average	53.22b	37.19a	7.44a	1.38	0.25	0.00
Single-stage[3]	Males	48.88	43.63	6.63	0.63	0.25	0.00
	Females	77.98	19.63	1.13	0.38	0.38	0.13
	Average	63.38a	31.63b	3.88b	0.50	0.31	0.06
	Males	41.44b	47.20a	9.44a	1.32	0.38	0,00
	Females	75.13a	21.63b	1.88b	0.57	0.18	0,06
Standard error		2.03	1.83	1.08	0.30	0.21	0.06
Source of variation		**P - value**					
Treatment		0.003	0.034	0.025	0.128	0.419	0.328
Sex		<0.001	<0.001	<0.001	0.051	0.885	0.328
Treatment x Sex		0.191	0.780	0.414	0.309	0.232	0.328
Farm [Treatment]		<0.001	<0.001	0.674	0.334	0.577	0.450

a-b Means within the same column of similar treatment without common letter are significantly different (P < 0.05). 1 Gait scores indicate normal gait (score 0) to immobile chickens (score 5), according to Kestin *et al*. (1992). 2 - Egg pre-warming for multi-stage machines was done in the room machine (25.6 a 26.7 °C) during 5 hours before they were placed inside machines at 37.5°C. The initial average temperature for hatchers was 36.9 °C and the final 36.7 °C. 3 - Egg pre-warming for single-stage machines was done directly inside the machines (26.7 °C) for 6 hours. Seven stages were used in the setters and 3 in the hatchers. The initial average temperature in the hatchers was 36.7 °C and the final was 36.1 °C. The total time of incubation was determined by the hatchery manager according to the chick condition and experience in machine management. The incubation time was approximately 509 h for single-stage machines and 505 hours for multi-stage machines.

Breeder nutrition, management, and incubation

In several experiments, we have evaluated combinations of breeder treatments and incubation conditions. In general, some breeder effects have been observed in the progeny, but the incubation effects seem to have stronger impacts on bone and tendon development, prevalence of leg disorders, locomotion issues and bone strength close to market age.

Breeder trace mineral nutrition and pre-warming

The addition of OTM (Mintrex® P, 1 kg/MT) additionally to the inorganic premix of Ross 708 broiler breeder diets from start of lay was evaluated (Oviedo-Rondón *et al*., 2008a). Eggs from the flock at 35 wk of age were pre-warmed in either a NON-UNIFORM condition at 23.9 °C without forced air movement for 9 hours or in UNIFORM condition at 26.7 °C with forced air movement for 11 hours. All eggs were thereafter incubated in a similar manner and exposed to elevated temperatures in the hatchers. Bone development was evaluated in 20 chicks per treatment at hatch. Chicks were grown to 49 days in 48 floor pens. Gait scores and leg disorders were observed at 40 days and incidence per pen was determined. Broilers were processed at 49 days, and both tibias were collected. Tibia mineral density (BMD) and bone mineral content (BMC) were determined with DEXA. Data was analyzed as a 2 x 2 completely randomized factorial design with breeder diet (Control *vs* OTM) and egg pre-warming (NON-UNIFORM vs UNIFORM) as main effects.

Results indicated that treatments did not affect chick body weight or yolk utilization at hatch;

however, UNIFORM egg pre-warming caused lower relative weights of femurs and tibias, shorter femurs and tibias, and higher RA of tibias and shanks. Chicks from breeders fed added OTM had thicker shanks and lower shank length RA. The incidence of twisted legs at 40 days was affected by both breeder diet and egg pre-warming treatments. UNIFORM egg pre-warming reduced incidence of twisted legs. Breeder diets with added OTM produced broilers with a lower ($P< 0.01$) incidence of gait score 2 or any gait score higher than 1. BMD and BMC were not affected by treatments, but the RA in BMD was reduced ($P = 0.08$) by added OTM in breeder diets. It was concluded that added OTM in breeder diets can improve bone development and gait scores in the progeny, and egg pre-warming conditions may affect bone development and minimize bone disorders in broilers.

Breeder age, egg storage time and egg pre-warming rate

In another study (Eusebio-Balcazar *et al*., 2009a), the effects of breeder age, egg storage time, and egg pre-warming rate were evaluated on bone development at hatch and leg health in broilers at 42 days. Fertile eggs from two breeder flocks 33 and 57 wk-old were collected and stored for 2 weeks, and again at 35 and 59 weeks, eggs were collected and stored for 3 days. Prior to incubation these eggs were warmed up to temperature in one incubator over periods of either 2 or 18 hours, and later incubated under the same profile. This arrangement of treatments resulted in a 2 x 2 x 2 factorial design with hen breeder age: old or young; egg storage, fresh or stored; and egg pre-warming rates, fast or slow, as main factors. A random sample of 10 chicks per treatment were collected at hatch, weighed, and sacrificed with residual yolk determined. Both legs were dissected; shank and femur weights, lengths, thicknesses, and ash contents were obtained. The RA and weight relative (%) to body weight without yolk of each leg section were calculated. A total of 1008 chicks were randomly placed in 72 floor pens (14/pen). At 42 days of age, chickens were gait scored and individually inspected for crooked toes, valgus/varus deformities, hock burns, and foot pad dermatitis.

Results indicated that heavier body weight without yolk, higher femur ash content, lower thigh weight and RA of shank length were observed on old breeders compared with young. Chicks coming from fresh eggs had lower RA of femur weight compared with chicks from stored eggs. Chicks had heavier femur relative weights, but lower ash content when egg pre-warming profile was slow compared with fast. A three-way interaction was observed for valgus, and egg storage by pre-warming profile interaction was observed in gait score 1 at 42 days. Broilers from young breeders and fresh eggs had lower incidence of valgus independent of warming profile, as compared with old, fresh, fast broilers (56 vs 78%). No differences were observed in the incidence of other leg issues. Breeder age, egg storage time, and egg pre-warming rate influenced bone development and leg problems in broilers.

Breeder restriction and incubation profiles

The development and strength of gastrocnemius tendon is crucial for broiler locomotion. Failure in the correct alignment, structure and size of collagen fibers and glucosaminiglicanos that are part of the tendon affects normal walking ability, lessens mobility and as a consequence affects bone development during life (Foutz *et al*., 2007). Our research group at North Carolina State University in collaboration with Dr. J. Halper from the University of Georgia evaluated the size of collagen fibers type I and decorin in tendons of two groups of chickens with different management at breeder level, incubated under two incubation temperature profiles. Breeders of the male line were either fed *ad libitum* or feed restricted, and then an additional control group of feed restricted breeders of the commercial cross was included in this evaluation (Oviedo-Rondón *et al*., 2010).

Eggs were incubated under either standard conditions to maintain an eggshell temperature close to 37.5 ºC, or with a temperature profile similar to the one similar to multi-stage machines where eggshell temperatures were kept at 36 ºC during the first week, 37.5 ºC in the second week and 39 ºC the last week of incubation. At hatching, males were selected and placed in battery cages until 21 days of age. Tendons were evaluated by histology at hatch, 4, 14 and 21 days of age, and bone relative weights, lengths and RA were also evaluated.

Results indicated that collagen fibers were thicker in tendons of chickens that were incubated under standard conditions compared to those chickens incubated under the multi-stage temperature profile in all age periods evaluated. Relative weights of bones were higher and bones were longer in chickens coming from the standard incubation profile. In this study, no consistent interactions with breeder treatments or differences due to feeding management in the breeders were observed. Results of this study indicated that incubation conditions have more importance on bone and tendon development than the feed restriction of breeders.

Breeder feed restriction programs and incubation profiles

As a preliminary evaluation of the effects of breeder feed restriction programs on progeny development and performance, two experiments were conducted to assess the effects of the skip a day and every day breeder feed restriction programs. This factor was evaluated together with two incubation temperature profiles, the standard or multistage profile (early low, late hot) in 54 and 60 wk-old Cobb 500 breeders, in experiments using a factorial design. Bone parameters similar to the ones described for the previous experiments were evaluated. These preliminary results indicated no significant interaction effects of treatments on bone development at hatch. In both experiments, incubation temperatures affected ($P<0.05$) relative weights of tibias, and shank length at hatch. Chicks incubated under standard temperature had longer shanks. Only in the second experiment were observed significant effects of incubation on RA of shanks relative weight and femur, tibia and shank length.

In the second experiment, broilers were grown to 21 days in battery cages and relative weights of bones and lengths were evaluated at the end of the experiment. Treatment interaction effects were observed on femur, tibia and shank relative weights and lengths. Progeny of breeders with every day feeding were not affected by incubation conditions, but chicken progeny of breeders under the skip a day feed restriction program had lower relative weights of bones and shorter bones when the standard incubation program was used. The interaction effect was also observed on RA of shank relative weights. Chicken progeny of breeders restricted fed with a everyday feeding had lower RA when incubation temperature was standard than when incubation followed the multi-stage profile, while no difference on RA was observed due to incubation in progeny from breeders fed following the skip a day program. The RA of femur length was also higher for progeny of breeders with skip a day program than with everyday feeding. Incubation treatments affected shank width and its RA, as well as RA of shank length.

In summary, this preliminary investigation indicates that breeder feeding programs may have carryover effects on bone development of broiler progeny. The effects could not be detected at hatch but were observed 3 weeks later. These observations need to be reevaluated due to welfare implications. These experiments confirm that incubation temperature profiles modifying early and late incubation affect bone development of broilers.

Conclusions

Breeder management and nutrition, and adequate incubation are important to diminish the prevalence of locomotion issues and leg disorders in broiler flocks. The research results presented indicated that changes in amounts of feed offered between 14 and 29 weeks and the changes in feeder space from rearing to laying phases can affect egg properties. The changes in breeder feeder space at photostimulation seem to affect the bone development of progeny during the embryonic period. Chickens that had more feeder space in the laying house had the worst gait scores at 6 weeks of age compared to chickens of breeders that maintain similar feeder space during the growing and laying period. Breeder feed restriction programs may impact chicken development, but feeding breeders *ad libitum* does not seem to affect progeny bone or tendon development. Avoiding long storage (more than 7 days), conducting adequate uniform pre-warming of eggs prior to incubation, and maintaining optimum eggshell temperatures during the entire incubation period, especially during the first days and in the hatchers, is critical for correct development of bones and tendons, and consequently, to diminish the prevalence of leg problems in broilers. Broiler nutrition and management are still important to minimize problems, but when certain aspects of embryonic development are altered, it is difficult for any nutritional or management intervention to have a positive effect. Under commercial conditions only 1 to 3% of broilers are severely affected by leg and locomotion issues. This indicates that the inadequate microenvironments in incubators causing stress to a small proportion of embryos may be an important factor affecting prevalence of leg problems in broiler flocks.

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