ABSTRACT

DA COSTA, MANUEL JOÃO GONÇALVES. Incubation and Nutritional Factors that Affect Footpad Skin Development and Dermatitis. (Under the direction of Dr. Edgar Oviedo-Rondón).

Footpad dermatitis (**FPD**) is an economical and welfare concern for poultry production. The objective of this study was to ascertain how incubation and nutritional factors affect the footpad skin development and severity of dermatitis of ducks and chickens. Stressful incubation temperatures profiles (**TEM**) were a common factor across the four experiments. Performance, residual yolk, footpad skin histological structure and FPD were evaluated. In the first experiment (Chapter II) it was observed that elevated TEM and reduced eggshell conductance did not have an effect on duck BW at hatch or 35 d, even though they had increased residual yolk at hatch. Footpad dermis and *papillae* height were affected by treatment interaction at hatch. At 35 d reduced conductance increased epidermis layer. Both, reduced conductance and elevated TEM, increased the probability of FPD in ducks at market age. The subsequent 3 experiments were performed in broilers and the aim was to evaluate the impact of temperature variations usually observed in multistage incubators, low temperatures in the early stages and elevated during the later (**LH**), in comparison with standard (**S**) TEM on footpad skin structure and FPD. The treatments of the second experiment (Chapter III) were a combination of two breeder feed restriction programs, every-day feeding vs. skip-a-day, with the two TEM. Broiler performance was negatively affected by the LH TEM at 7 and 21 d. On footpad skin structure *stratus corneum* (**SC**), dermis and *papillae* height were affected by treatments on d old chicks. Dermis was also increased on skip-a-day progeny. At 7 and 22 d a reduction on dermis layer was observed on LH chickens. The third experiment tested the effect of TEM and two trace mineral sources, inorganic vs. organic, with reduction of the inclusion levels of the OTM source, on footpad skin development and collagen

content. At hatch, LH TEM increased the BW of chickens in comparison with S TEM. However, it was observed that this effect was associated with higher residual yolk present in LH chickens. Furthermore, S TEM treatments reduced FCR of chickens at 21 d. At hatch, the S TEM treatments increased dermis development. Additionally, S TEM also increased SC length at 7 d. The dietary organic diets with reduction of inclusion levels increased dermis parameters at 21 d. No effects of treatments were observed on collagen quantification. The fourth experiment was intended to evaluate if the effects on skin development observed on the previous experiments had influence on FPD when the birds were under simulated commercial conditions. Therefore, male and female chickens incubated both with S or LH TEM were housed and evaluated for FPD severity. The histological analysis demonstrated no effects of TEM on footpad structure. The FPD evaluation showed that LH TEM chickens had higher probability to develop FPD lesions than chickens incubated under S TEM at 13 d. However, when evaluating FPD at 28 d the effect of TEM treatments was no longer observed, being the FPD lesions strongly related with litter moisture. Nevertheless, chickens incubated with LH TEM were more susceptible to develop severe FPD lesions when litter moisture increased.

In conclusion, it was demonstrated that incubation conditions affect duck and broiler performance and have a role in skin and FPD development. The exposure of the embryo to improper temperatures mainly affected dermis structure at hatch and increased bird susceptibility to litter moisture increasing the probability of birds to develop FPD. Furthermore, parameters such as parent stock nutrition management and dietary trace mineral sources also affected dermis footpad skin development which may also be related with higher tendency to develop FPD lesions.

Incubation and Nutritional Factors that Affect Footpad Skin Development and Dermatitis

by
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DEDICATION

To my brother, Manuel António Da Costa; to my parents, Manuel Fernando Da Costa and Maria de Fátima Gonçalves; to my aunt, Maria de Lourdes Gonçalves; to my grandparents, Julia Teixeira, Adelino Gonçalves, António Da Costa, and Rosalina Moreira. This achievement was only possible because you were there to support me and helped to walk strong through the path of my life.

To my girlfriend, Bianca Lourenço, for always being there when needed, for being the best companion that I could have, for the advices, for the friendship, for all the love. Thank you for everything. You were and you will always be my best friend. I can foretell a bright and happy future for us. We go and we see!

BIOGRAPHY

Manuel G. da Costa was born on June 15th, 1986, in Porto, Portugal. He obtained his Veterinary degree and Masters in Veterinary Medicine at the ICBAS – Universidade do Porto. During his veterinarian studies, he was involved in the direction of the National Veterinary Internships and other Veterinary student association projects. Additionally, he participated in several internships mainly related with animal production. He got his first field experiences with poultry at Lusiaves Inc. in Portugal, in the broiler production department. Afterwards, he travelled to USA where he was an intern at the Poultry Science Department of North Carolina State University, under Dr. Edgar Oviedo's supervision. He also had the opportunity to experience one month of the Master in Avian Medicine program at University of Georgia. After these internships, Manuel travelled to Brazil where he obtained field experience in two poultry companies, Pluma Agroavícola and Frangos Canção. In order to pursue further education he decided to work again in the USA where he worked for Dr. Oviedo 6 months and started his Master of Science at the Poultry Science Department in August 2011. He has conducted research focused on nutrition, incubation, and leg and footpad health of broilers, turkeys and ducks. During his master program Manuel presented and was co-author of presentations at several scientific and industry poultry meetings. In 2012, Manuel was awarded with the Jones-Hamilton at Poultry Science Association Annual Meeting and participated in the World's Poultry Science Student Program at Bahia, Brazil. After his graduation Manuel will continue his poultry studies and pursue a Doctor of Philosophy degree at University of Georgia under Dr. Gene Pesti.

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TABLE OF CONTENTS

LIST OF TABLES

CHAPTER I

CHAPTER II

CHAPTER III

CHAPTER IV

CHAPTER V

LIST OF FIGURES

CHAPTER I

LITERATURE REVIEW

INTRODUCTION

Being the world leader in poultry production, the United States of America is responsible for producing around 16,290,117 tons of ready-to-eat poultry meat each yr (USDA-NASS, 2013). Chicken meat leads the list of most produced poultry meat, followed by turkey and duck products (USDA-NASS, 2013). In the past yr the processing and selling of poultry in cut parts became a common practice mainly due to preferences of consumers and the economics of aggregate extra value to the product (USDA-ERS, 2013).

The parts most demanded by the market are the breast, wings and paws (US Poultry & Egg Export Council, 2009). Even though the majority of the American population does not consume paws, the exportation of this product to Asian markets brings significant revenues to the companies, making it an essential source of profit for poultry companies. Approximately 20 yr ago, this product was valueless and ultimately was used for rendering. Nowadays, USA exports around 300,000 metric tons of paws every yr, representing a yearly estimated income of $280 million (US Poultry & Egg Export Council, 2009; Philips, 2011).

The value of a paw in the market is determined by its size and quality, with the quality being defined by its overall integrity and health. At processing, this type of product is inspected federally under the Finished Product Standards (Shepherd and Fairchild, 2010). The grading of the paws in the plant occurs post-scalding and chilling and takes into account the presence of inflammatory processes, wounds, bruises, fractures, extraneous material, cuticle losses and mutilations (USDA-FSIS, 2012). Considering these parameters, the plants usually classify paws into A, B or condemn categories. The size of paws is also a determinant of the value of the product, with the

paws being grouped and priced according to three categories: small, medium and large-jumbo (Shepherd and Fairchild, 2010). The large-jumbo size is preferred by the Asian markets, for which it became incentivized by the poultry industry (Skrzycki, 2010).

The majority of paw downgrades is due to the presence of footpad dermatitis (**FPD**). It is estimated that 99% of the condemnations come from FPD lesions (Shepherd and Fairchild, 2010). This type of lesion is a common finding in today's commercial poultry production (Berg, 1998; Breuer et al., 2006). Aside from the losses observed at processing plant, reduction of animal performance at the farms has also to be considered. Reduced performance of broilers and turkeys which present severe FPD has been reported by several authors (Martland, 1984; Martland, 1985; Ekstrand and Algers, 1997). This negative effect on animal performance was linked with painful gait conditions induced by the extreme inflammatory footpad lesions (Schmidt and Lüders, 1976; Mayne et al., 2007a). Impairing poultry mobility results in reluctance to move, negatively affecting feed consumption and consequently the growth rate. Furthermore, when birds sit for extended periods on the litter, the incidence of hock burns and breast blisters increases leading to higher rates of carcass condemnations and compromising the salubrity of the final product (Martland, 1985; Greene et al., 1985; Olivère et al., 2011). Also, it is hypothesized that footpad lesions may be an open door for pathogens that can eventually enter into the bloodstream, leading to systemic infections which can create food safety concerns in the industry (Hester, 1994; SCAHAW, 2000).

In addition to the mentioned economic impact, the stress and pain induced by FPD raise animal welfare concerns. The changes reported in walking ability related to FPD prevent the birds from expressing their normal behavior. According to Beaumont et al. (2007), animal welfare measures should guarantee that animals are free from hunger,

thirst, discomfort, pain, injuries, diseases, fear, and distress, and that animals are able to express a normal behavior, which might not occur under severe FPD conditions. The FPD incidence in poultry flocks is currently a criterion used in company welfare audits in Europe and United States (Berg, 2004; Berg and Algers, 2004; National Chicken Council, 2010).

Considering that paw quality, and specifically FPD, have such an important role on animal welfare and sustainability of the companies, there is a need to understand all the factors that are involved in and can ease the severity and incidence of this condition. The following section will review common factors reported to be involved on FPD etiology and severity, and also describe some alternative factors that might influence footpad skin development and create individual susceptibility of the birds to develop FPD.

LITERATURE REVIEW

Footpad Skin Structure and Development

Footpad skin of poultry is considered a specialized epidermal type that protects birds against mechanical and chemical agents present in the environment (Michel, 1992). The paws of poultry species are covered with two distinguished types of scale (Sawyer and Knapp, 2003). The dorsal side of the metatarsus and toes are covered with scutate scales while the plantar surface of feet is covered with reticular scales (Prin et al., 2004). The scales are arranged in a tight non-overlapping pattern, having a symmetrical papillary structure (Prin et al., 2004). Histomorphologically, these *papillae* have three major layers: the *stratus corneum*, epidermis and dermis (Figure I - 1) (Bacha Jr. and Bacha, 2000). Footpads are distinct from other skin tissues for having a thick *stratus corneum* and dermis and a cushion of fat subcutaneously (Bacha Jr. and Bacha, 2000). Skin development starts in the early stage of embryo differentiation, and by the end of 9^{th} d of incubation the anterior shank of the chicken embryo is covered with a flat two-layered epidermis (Sawyer, 1972; Bellairs and Osmond, 2005). The formation of scales starts around the 12^{th} d of incubation and is completed by d 16 (Sawyer, 1972; Gonzales and Cesario, 2003; Bellairs and Osmond, 2005). During this period each scale gets increased, keratinized, and blood vessels appear in the *papillae* (Bellairs and Osmond, 2005).

Footpad Dermatitis

The FPD lesions have been reported to affect chickens, turkeys, and ducks (McFerran et al., 1983; Mayne et al., 2005; Gibson, 2007), although no study has compared the lesions between species (Mayne et al., 2005). Macroscopically, the degree of extension

and severity of lesions vary, ranging from soft skin discoloration to deep ulceration (Mayne et al., 2005). In the early stages, keratosis and erosions are observed, being followed by small, scaly, brown scabs that can progress to cracking and ulceration (Santos et al., 2002; Mayne et al., 2005; Allain et al., 2011). Concomitantly, swelling, hyperplasia, epidermis necrosis and secondary bacterial infections may be observed (Greene et al., 1985; Pass, 1989).

Histomorphologically, the major lesion observed is a hyperplasia of epidermis with the individualization of keratin layers (Pass, 1989; Platt et al., 2001). This acanthosis creates hyperkeratosis and consequent papillary growth (Pass, 1989). During the early phases of life (13 d) Santos et al. (2002) reported a hydropic degeneration of epidermis and signs of extreme inflammation in the epidermal-dermal junction on broilers with FPD. In the advanced stages of lesions, the *stratus corneum* also shows evident defects with thickening and formation of 'horned pegs' (Martland, 1984; Whitehead, 1990). These lesions are also followed by an acute lymphocytic and granulocytic response with concomitant dilation of blood vessels (Martland, 1984, 1985; Greene et al., 1985; Whitehead, 1990; Santos et al., 2002).

The FPD develops fast under commercial conditions, and some signs can be observed as early as four d of age (Breuer et al., 2006). Considering this, the lesions are observed during all the life stages of the birds, tending to worsen in severity and size with age if improper management is provided (Mayne et al., 2006; 2007a). Several methods have been proposed to evaluate the prevalence of FPD in the industry creating difficulties when assessing the extent to which this condition affects the flocks (Arnould, 2011). However, it is recognized as a well-spread and variable problem in poultry production (Berg, 2004; Bessei, 2006; SCAHAW, 2000).

The etiology of FPD is still not completely understood. A multifactorial origin of FPD have been reported, with litter conditions being a major causative agent (Shepherd and Fairchild, 2010; De Jong et al., 2012). Other factors such as management, nutrition and genetic background also play an important role in FPD development and should be regarded (Mayne, 2005; Mayne et al., 2007a, Shepherd and Fairchild, 2010; Wu and Hocking, 2011; De Jong et al., 2012).

Litter. Litter conditions have been reported as the number one factor on the onset of FPD (Martland, 1984; 1985; Shepherd and Fairchild, 2010; Youssef et al., 2011; De Jong et al., 2012). Considering that animals spend most of their time in close contact with litter, any factor that affects litter quality will have an impact on the development of this type of contact dermatitis (Eichner et al., 2007; Bilgili et al., 2009). Physical structure, moisture, ammonia and other chemical substances will determine the quality of the litter and influence paw health (Mayne, 2005). Variables such as type of bedding material, stocking density, ventilation, down time, type of drinkers, season and fecal viscosity are involved in litter quality definition (Shepherd and Fairchild, 2010).

Each bedding material type has different water absorbance capacity and physical structure that can influence the prevalence and severity of FPD in broiler chickens and turkeys (Bilgili et al., 2009, Youssef et al., 2010). In three consecutive trials, Bilgili et al. (2009) evaluated the effect of pine shaving, pine bark, chipped pine, mortar sand, chopped wheat straw, ground hardwood pallets, ground door filler, and cotton-gin trash as bedding materials over time on FPD incidence in broilers. Mortar sand and door filler resulted in less incidence of FPD over the three trials. Chipped pine and chopped wheat

straw resulted in the highest incidence of lesions in all three trials, this being more evident in the first trial. The incidence of FPD increased consecutively in each trial across all the bedding materials. Pine shavings and cotton-gin trash, when reused between flocks, tended to increase FPD. In another three broiler experiments, Grimes et al. (2006) tested pine shavings and aGroChips®, an alternative bedding material made from chopped cotton lint waste, gypsum, and old newsprint, on footpad score. No differences were observed on footpad conditions, though bedding material type had a significant effect on litter quality.

In a similar experiment, Youssef et al. (2010) observed an effect of litter material on footpad score in turkeys. Wood shaving, lignocellulose (SoftCell®), chopped straw (Strohfix®) and dried maize silage were used as four litter material treatments over a 4 weeks period. Additionally, half of the turkeys on each litter were exposed to wet litter for 8 h each d. Differences in footpad scores were observed between litters with these effects dependent on moisture exposure. Lignocellulose litter demonstrated better condition for footpads both in dry and wet litter. Under dry litter conditions, chopped straw had the highest severity of FPD. Litter moisture increased FPD scores across all the litter types.

As observed in the experiment described above, litter moisture is a great contributor to the development of FPD. In four experiments Youssef et al. (2011) tested the effect of exposing 2-wk-old female turkeys to wet litter for 8 h per d. Across the four experiments high litter moisture potentiated the prevalence and severity of FPD. These authors argue that exposure to high litter moisture for a limited period of time is enough to induce FPD, and that litter moisture is the major contributor to FPD condition. The same relationship between litter moisture and FPD was observed by other authors (Martland 1984; 1985;

Ekstrand et al., 1997; Eichner et al., 2007). Furthermore, Mayne et al. (2007b) observed that 48 h of exposure to wet litter was enough to trigger inflammatory response in the footpads. Despite the consistency of results in the relationship between litter moisture content and FPD development, Cengiz et al. (2011) did not observe significant differences on footpad scores between dry and wet litter. Nevertheless, other factors that affect litter moisture, including drinkers design and management, stocking density, ventilation equipment and management, enteropathies and nutrition parameters should be evaluated to prevent FPD.

Season. It has been reported there is a seasonal variation on FPD incidence. Overall, it is argued that this difference between seasons is mainly linked to environmental factors which affect litter moisture (Shepherd and Fairchild, 2010). Several researchers reported an increase of FPD incidence in cold weather (Ekstrand and Carpenter, 1998; Haslam et al., 2007; De Jong et al., 2012). Haslam et al. (2007), argued that this relationship between cold weather and FPD is probably a result of bad litter management due to the low ventilation rates used to maintain house temperature. In a study conducted in Netherlands, De Jong et al. (2012) observed that broiler flocks raised during months with high environmental temperatures had lower FPD scores than broilers from months with colder temperatures. Alternatively, Wang et al. (1998) observed a different effect of environmental temperatures on FPD. These authors reported that when White Leghorn chickens were exposed to high environmental temperatures the incidence of FPD was higher than when they were subjected to low temperatures. Although, it should be considered that in this experiment the Leghorn chickens were only placed in the litter after 13 wk of age and under a low animal density scheme ($6/m^2$). Nevertheless, the

environment clearly influences litter conditions and consequently may have an impact on FPD development.

Inheritance and Breeder Nutrition Management. Parent stock genetic background and management are parameters that were reported to have an influence on the incidence of FPD (Breuer et al., 2006; De Jong et al., 2012). Differences between genetic lines and the potential for genetic selection to minimize FPD severity on the farms were reported among poultry species. Several authors argue a potential for genetic selection to reduce FPD incidence together with selection for BW increase (Kaejer et al., 2006; Ask 2010; Kappel et al., 2012). Differences in FPD severity among five fast-growing genetic lines were observed recently by De Jong et al. (2012). In this survey, Hubbard Flex had the lowest level of FPD and Ross male had the highest scores. Additionally, Bilgili et al. (2006) found that the severity of FPD in two strains-crosses was different under different density diets suggesting that FPD is dependent on the genetic background. Breed effects were also reported on turkey poults. Chavez and Kratzer (1972) argued that Large White turkey poults were more susceptible to FPD than Broad Breasted Bronze. These authors suggested this may be due to the high growth rate of Large White in comparison with Broad Breasted Bronze.

The nutrition and management during rearing and reproduction phases of poultry parent stock have been reported to impact progeny performance and development (Romero, 2008; Eusebio-Balcazar, 2010). In broiler breeder production, limiting the feed intake has been a common practice to avoid overweight breeders, increase egg production and increase livability (Rosales, 1994; Beer and Coon, 2009). Usually the usage of skip-a-day restriction programs is preferred over the daily feeding to promote the uniformity of

the flock (Beer et al., 2007). The usage of these types of programs can induce changes in lipid metabolism, increasing the lipogenic activity of the liver (Rosebrough, 2000; Richards et al., 2003), which will influence the fat deposition in the eggs (Cherin and Sim, 2001; Beer and Coon, 2009). Since embryo metabolism is around 90% fueled by lipid consumption (Romijn and Lokhorst, 1960; Donaldson, 1981) any change in this component may alter its development. Furthermore, it has to be take into account that fatty acids are also needed for the synthesis of phospholipids for membrane formation and triglycerides for energy storage (Donaldson, 1981).

Hen feeding regime and management have been shown to impact progeny performance and body structure. When hens were subjected to feed restriction programs, their offspring were reported to have lower tibial bone breaking force and BW at hatch and market age (Triyuwanta and Nys, 1990; Triyuwanta et al., 1992). Effects of breeder management on FPD incident were also observed by Breuer et al. (2006). When comparing footpad condition of poults coming from hens on the 4^{th} and 20^{th} week of production, the progeny of the older breeders had poorer footpad development with signs of inflammation at 7 and 21 d. The authors argue that this could be due to higher BW observed at 7 d in the poults coming from the older breeder. Nevertheless, based on these results, FPD severity may be influenced by breeder parameters.

Incubation. Despite sometimes being disregarded by the industry, the incubation period is an essential phase on poultry life cycle (Lindgren and Altimiras, 2011). Recently De Jong et al. (2012) observed that hatchery conditions had an effect on the incidence of FPD. Even though no clear trend was observed on incubation parameters and FPD, the

differences reported by De Jong et al. (2012) clearly demonstrated the existence of unknown factors that do influence the incidence of this condition.

The environmental conditions and stress stimulus that some embryos may be exposed to during incubation will reflect on embryo size, organogenesis, physiological development and hatchability (Decuypere and Michels, 1992; Yalçin and Siegel, 2003; Tazawa et al., 2004; Oviedo-Rondón et al., 2009; Molenaar et al., 2011). Swayer et al. (1984) reported that the morphogenesis of skin, feathers, spurs, reticular and scutate scales are impacted by the epidermis-dermis interaction during embryo development. Consequently, any incubation parameter that affects embryo development may affect skin and its appendages development.

Several authors have reported effects of incubation temperatures on embryo development (Leksrisompong et al., 2007; Yalçin et al., 2008). In an experiment conducted by Yalçin et al. (2008), high temperatures (38.5°C) between the 10^{th} and 18^{th} d of incubation were reported to increase BW at hatch but prejudice heart and liver weight. An interesting finding observed was that high incubation temperatures had a negative effect on beak development at 18^{th} d of incubation. Even though no differences were observed between the standard and high incubation temperatures treatments at hatch, based on the effect observed at the 18^{th} d the stressful conditions during incubation may have an effect on keratinaceous tissue development.

Concomitantly with the effects in body structure and composition, changes in hormonal and metabolic regulations are also reported (Moraes et al., 2004; Yahav et al., 2004; Piestun et al., 2009). Piestun et al. (2009) observed that high incubation temperatures (39.5°C) between d 7 and 16 of incubation reduced the T_3 plasma concentration. Similar effects on T_3 and T_4 plasma concentrations were described by

Christensen et al. (2005) when turkey embryos were exposed to high temperatures (38 and 39°C) during the last four d of incubation. Nevertheless, the effects on thyroid hormones concentration in the plasma are not consistent and may depend on the level, duration and age where embryo is exposed. For example, Yalçin et al. 2008 observed that when embryos were exposed to high temperatures (38.5°C) between d 10 and 18 of incubation had higher levels of T_3. Effects on stress hormones levels were also noted when eggs were exposed to elevated temperatures. (Iqbal et al., 1990; Moraes et al., 2004). Moraes et al. (2004), demonstrated that embryo blood corticosterone during incubation may be increased due to heat stress situations.

Changes in thyroid and adrenal hormones were reported to negatively affect skin thickness in humans and animals, creating disease conditions. Hyperadrenocorticism is strongly associated with thinner skin in Cushing's disease (Sheppard et al., 1966; Safer et al., 2003). Furthermore, several authors have demonstrated a negative effect of cortisone and hydrocortisone on scale morphogenesis in chickens (Moscona and Karnofsky, 1960; Stuart et al., 1972; Fisher et al., 1984). Fisher et al. (1984) showed that all types of scales were suppressed when embryos were exposed to doses of 40 µg of hydrocortisone at d 10 of incubation. Furthermore, reticulate scales were specifically absent on the most ventral part of the footpad when embryos were exposed to high doses of hydrocortisone (Fisher et al., 1984).

Another parameter that should be regarded during incubation is hypoxia conditions. Several researchers had argued that the availability of oxygen influences organ and embryo development (McCutcheon et al., 1982; Stock et al., 1983). Oviedo-Rondón et al. (2008a; 2008b) reported that elevated incubation temperatures and low oxygen concentrations negatively impact bone development in broilers and turkeys. In another

study Wineland et al. (2006) indicated that when chickens are exposed to high temperatures during the late period of incubation, hypoxia conditions worsen intestinal maturation. Dzialowski et al. (2002) found that embryos exposed to hypoxia after d 12 of incubation had a shorter beak at 18^{th} d of incubation demonstrating that keratinaceous tissues development are also affected by this parameter. Considering the findings of De Jong et al. (2012) and the above mentioned hatch parameters that may affect the development of footpad skin, incubation conditions should be considered as possible predisposing factors for FPD.

Nutrition. Together with litter conditions, nutrition has been considered a major parameter in initiating FPD (Shepherd and Fairchild, 2010). Diets deficient in biotin, panthotenic acid, riboflavin, methionine, lysine and zinc have been reported to be involved in skin development and influence FPD development (Lepkovsky and Jukes, 1936a,b; Kratzer and Williams, 1948; Chavez and Kratzer, 1972; 1974; Murillo and Jensen, 1976; Whitehead, 1990; Buda, 2000; Hess et al., 2001). Furthermore, the inclusion of some ingredients when formulating poultry diets can induce changes in fecal moisture and viscosity, negatively impacting litter quality. Some of these ingredients and nutrients are soybean meal, non-digestible fat, wheat, energy, protein, sodium, chloride, and potassium (Bradshaw et al., 2002; Mayne, 2005; Shepherd and Fairchild, 2010; Arnould, 2011). Nevertheless the nutritional parameters described to have an impact on FPD are:

Dietary energy density - The dietary energy density is mainly related to the portion of energy coming from fat sources. Consequently, diets with low fat inclusion are

considered to be low energy density and with high fat inclusion are considered to be high energy density (Shepherd and Fairchild, 2010). Bilgili et al. (2006) observed that high density diets (Table I – 1) induced higher FPD scores in broiler chickens and that this effect was dependent on the chicken strain. The fat inclusion, composition, source and quality influence overall fat utilization and fecal moisture and viscosity (Collett, 2012). Unsaturated fatty acids have better solubility, which makes them more available to birds in comparison with saturated fatty acids (Baião and Lara, 2005). Consequently, diets with low digestible fat sources will produce sticky feces with high fat content that will negatively impact litter conditions (Bray and Lynn, 1986). Also, the usage of saturated fatty acids rather than unsaturated fatty acids leads to higher water intake, which can lead to high litter moisture content (Collett, 2012). Another factor to take into consideration when using high fat levels in the diets is the age of the birds. The lack of bile salts production during the early stages of life of the birds prevents them from digesting fats with high proportion of saturated fatty acids (Collett, 2012). Considering these parameters, any nutritional formulation strategy that increases fecal fat content, and consequently moisture, should be contemplated in order to avoid wet litter and FPD.

Protein - The protein sources used in poultry diets can be from animal, vegetable and synthetic origin. Diets with totally vegetable protein sources are becoming more common than diets with animal sources worldwide (Collett, 2012), with soybean meal being the most abundant and common source used (Nagaraj et al., 2007a). Some authors have shown that the inclusion of soybean in poultry diets has a negative effect on poultry feet quality by increasing FPD (Abbott et al., 1969; Jensen et al., 1970; Nagaraj et al., 2007b; Eichner et al., 2007). The increment in the incidence of FPD is thought to be related to its

low content in biotin and to its negative impact on fecal moisture (Mayne, 2005). Soybean meal has several anti-nutritional factors that can reduce digestibility, and increase excreta viscosity and consequently litter moisture. Non-digestible carbohydrates such as sucrose, stachyose, raffinose, and cellobiose, are present in great amounts in soybean meal and can reduce digestibility and increase the quantity of mucous in excreta (Leeson and Summers, 2001). Also, the presence of these non-starch polysaccharides can sequester proteins, which can lead to fermentation in the hind-gut promoting the development of dysbacteriosis and flushing (Collett, 2009). The usage of soybean meal also has the downside of being naturally deficient in biotin which, together with the capacity of produce sticky and high pH feces, can increase FPD (Abbott et al., 1969; Jensen et al., 1970). This increment in pH and moisture creates the optimum conditions for urolytic bacteria in the litter to convert excreted uric acid nitrogen into ammonia. The high fecal pH and ammonia content lead to an extreme alkaline environment that can chemically irritate the footpads (Abbott et al., 1969; Jensen et al., 1970).

Besides the impact of high inclusion of soybean meal, high protein diets *per se* have been related to high incidence of FPD (Table I – 1). High protein diets can increase nitrogen excretion, leading to high ammonia levels in the house which is related to paw burns (Nagaraj et al., 2007a,b). Furthermore, diets with excessive protein require an increase in water intake to excrete the excessive nitrogen, leading to feces with high moisture content (Gordon et al., 2003; Francesch and Brufau, 2004). In a study conducted by Nagaraj et al. (2007b), no difference on FPD severity was observed between diets with high and low protein although the litter total NH_3-N levels were higher in high protein diets. It was also noted that the severity of footpad lesions was higher when diets were all vegetable based when compared with diets that contain both vegetable and animal

sources. In another experiment, Nagaraj et al. (2007a) reported the same effect of protein source and inclusion on FPD. However, in this experiment the authors observed a significant difference on FPD severity between low and high protein diets. Severe lesions increased from 10% of incidence on birds fed low protein diets to 21% on birds fed high protein diets. Another dietary nitrogen related concern on FPD is methionine deficiency. Methionine was reported to reduce FPD in young poults, though it was observed that this effect was strain dependent (Chavez and Kratzer, 1974). Also Murillo and Jensen (1976) observed the same effect of methionine deficient diets and that the supplementation of cysteine and potassium sulfate did not improve footpad condition.

Enzymes - The use of exogenous enzymes as a potential measure to reduce FPD has been reported (Nagaraj et al., 2007b). In poultry, especially at younger ages, the production of endogenous enzymes is limited (Krogdahl et al., 1989; Sklan, 2001). Worldwide, the usage of soybean meal, wheat, and barley is common in poultry diets. These types of grains have high content of non-starch polysaccharides that can reduce diet digestibility, increase the amount of mucous in excreta and promote enteritis (Collett, 2009; Leeson and Summers, 2001), which can increase litter moisture. The addition of exogenous enzymes such as xylanase, β -glucanase, cellulase, pectinase and amylase can promote the degradation of the anti-nutritional factors reducing the negative effects on fecal moisture content and digestibility (Cengiz et al., 2012a). Furthermore, the usage of enzymes in corn-soybean diets has been recently linked with beneficial effects on animal performance (Masey O'Neill et al., 2012).

Nagaraj et al. (2007b) reported reduction in FPD severity, at market age, with the inclusion of enzymes in all-vegetable diets. When animal by-products were included in

the diet, no beneficial effect was observed on FPD lesions. On the other hand, Cengiz et al. (2012a) did not observe any significant effect of the inclusion of an enzyme mixture (xylanase, β-glucanase, cellulase, pectinase, amylase, and mannanase) in barley-based diets on the reduction of this condition. Overall, usage of enzymes should be evaluated in order to improve diet digestibility and reduce fecal moisture which consequently can reduce FPD.

Trace Minerals - Trace minerals such as zinc, copper, and manganese are involved in skin structure and protection (McDowell, 2003). Collagen synthesis, keratinization and cell replication require zinc as a cofactor, and severe dermatitis due to its deficiency has been reported (Sunde, 1972; McDowell, 2003). Collagen and elastin cross-linking also requires copper as an essential cofactor to guarantee skin integrity (Zhao et al., 2010). Manganese also plays a role in animal structural development, being involved in anti-oxidant functions and bone development (McDowell, 2003).

Several studies evaluating the impact of inorganic and organic sources of these minerals on FPD have been conducted. In an experiment conducted in dwarf and normal laying hens, Burger et al. (1984) did not find any reduction on FPD condition when feeding diets with 50% more of the inorganic mineral premix than normal. They also observed that increasing 50% more of zinc and copper or iron, iodine, copper and cobalt did not ameliorate footpad lesions. Despite the previous findings, some authors have reported that these same minerals, when fed in organic form, may have a positive effect on footpad and skin health. This difference is discussed to be related with better absorption of the chelated minerals in comparison to the inorganic which have more antagonistic reactions with other dietary compounds in the gastrointestinal tract (Zhao et

al., 2010). Hess et al. (2001) observed reduction of footpad lesion scores in broiler females when fed diets supplemented with zinc chelated with amino acids. The authors also reported that this difference was not present in broiler males. Similar findings of organic zinc effect were reported by Saenmahayak et al. (2010) in a mixed sex experiment. When feeding diets with 50% of zinc coming from an organic complex the authors observed a reduction on FPD incidence and severity at 48 d of age. Furthermore, no differences were observed in the incidence of FPD when comparing diets containing 50% more inclusion of zinc as organic source with the diets containing half organic and half inorganic at normal levels. In another experiment, Zhao et al. (2010) compared the effect of zinc, manganese and copper as organic sources with the same minerals as inorganic sources on FPD development. In this experiment, the diets with chelated minerals had half the inclusion levels of zinc and manganese from inorganic sources (40ppm vs. 80ppm of Zn and 60ppm vs. 120ppm of Mn). The severity of FPD was higher with diets with inorganic source and organic sources increased the percentage of healthy paws. In two fields trials conducted by Manangi et al. (2012), the same trends were observed with the inclusion of organic and reduction of trace minerals. In one trial, these authors evaluated two diets: one with the inclusion of 100ppm inorganic zinc and the other one with 30ppm of zinc chelated with 2-hydroxy-4-(methylthio)butanoic acid (HMTBA). The organic diet had higher percentages of low scores on FPD than the inorganic diet. In the second trial, treatments consisted of two diets: one had the inclusion of 100ppm zinc, 125ppm copper and 90ppm of manganese of inorganic source, and the other diet with the inclusion of 32ppm zinc, 8ppm copper and 32ppm of manganese chelated with HMTBA. Again, in this trial the organic source had higher percentage of lower scores.

Biotin - Biotin is a vitamin needed for vital basic functions (Mayne, 2005). Its deficiency has been correlated with FPD (Whitehead, 1990; Whitehead and Bannister, 1981; Buda, 2000). Whitehead and Bannister (1981) reported that when fed biotin deficient diets, broiler chickens developed more feet lesions. This deficiency seems to be especially important in turkeys (Whitehead, 1990). Buda (2000) demonstrated differences in footpad structure when turkeys were fed diets with normal and high levels of biotin. Turkeys that received normal doses of biotin had a rough, uneven footpad surface with gaps between *papillae*. The supplementation of biotin improved footpad health, presenting smooth footpads with proper *papillae* formation in these turkeys. In spite of the evidence presented above, some studies contradict the role of biotin on footpad health (Chavez and Kratzer, 1972; 1974; Cengiz et al., 2012b). Recently, Cengiz et al. (2012b) did not observe any improvement of footpad integrity when comparing diets supplemented with 0.05, 0.1, 0.2 and 0.4 mg/kg of biotin.

Animal Factor. In poultry production, there are several intrinsic animal parameters that have been related to footpad lesions. Bird sex was reported to be one of these parameters, though there is a lack of experimental consistency about it. Some authors reported an increased predisposition in males to develop FPD in comparison with females (Greene et al. 1985; Bilgili et al., 2006; Nagaraj et al., 2007a). Other authors did not observe any effect of sex on FPD development (Ekstrand and Algers, 1997; Martland, 1984). Furthermore, in some experiments, females had higher incidence of FPD in comparison with males (Kjaer et al., 2006). In fact, female skin was reported to be weaker than males due to a protein matrix less dense, with less collagen and protein (Mayne,

2005). Furthermore, Bilgili et al., (2006) observed an interaction between strain cross and sex, arguing that the non-consistent results between sexes may be due to the usage of different strains.

Other internal factors that were reported to be related to FPD are BW and age. As birds age, BW increases with concurrent increment of pressure over the feet, which has been reported to increase the susceptibility to develop skin lesions (Shepherd and Fairchild, 2010). Hocking et al. (2008) reported that FPD is positively correlated with BW gain. Eichner et al. (2007) also observed worsening of footpad scores with age and BW gain. Interestingly, Cengiz et al. (2011) observed that after 52 d of age, the susceptibility of broiler chickens to develop FPD decreases even when under wet litter conditions. Recently, De Jong et al. (2012) reported that older flocks evaluated at the slaughter plant had less severe lesions of footpad than younger ones. Also, no correlation between weight and footpad lesions was reported by Martland et al. (1984) in turkeys.

Hypotheses and research objectives

In view of the importance of FPD on poultry welfare and company profitability, it is essential to clarify the origin of FPD and all possible factors that might be involved in it. Considering its multifactorial etiology, our goal was to study both well-known and alternative factors that might be involved in FPD. Additionally, we questioned why we observe, in flocks with the same genetic background, management, nutrition and litter conditions, some birds with severe lesions and others with footpads in perfect condition. For that, we hypothesized that some factors, such as incubation, breeder management and dietary factors can affect footpad development and may explain the variability of FPD

lesions within a flock. In order to accomplish our research goals, four experiments were conducted to test specific parameters that could affect footpad skin structure and increase FPD.

As a first approach we decided to perform three factorial experiments with incubation treatments as common factors:

1. Effects of eggshell conductance and incubation temperatures on duck footpad development. (Chapter 2)

2. Breeder feeding restriction programs and incubation temperatures effect on progeny footpad development. (Chapter 3)

3. Effects of incubation temperatures and trace mineral sources on footpad skin development. (Chapter 4)

Based on the three previous experiments, an experiment was performed to clarify if the changes on footpad development observed in the first experiments had any relevance on FPD incidence and severity under simulated commercial conditions:

4. Effect of incubation temperature profile on broiler FPD.

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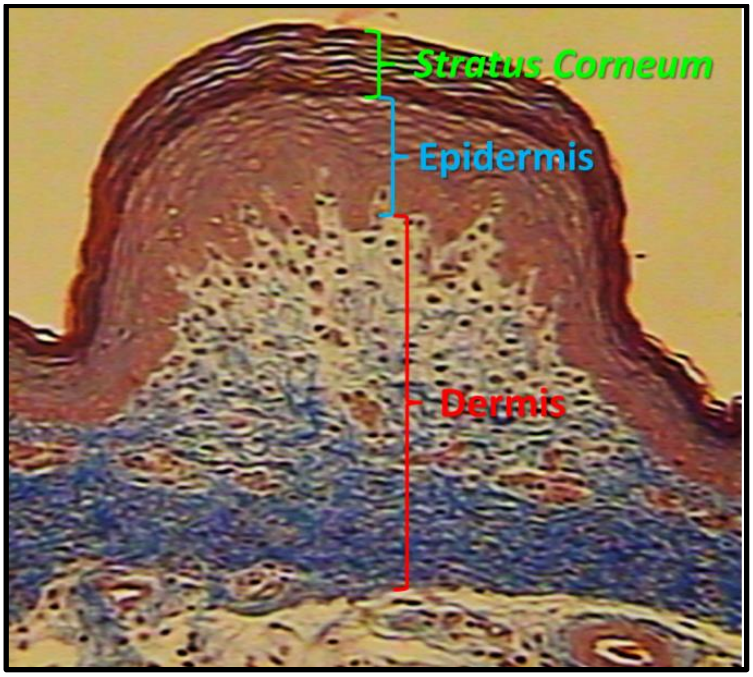

Figure I – 1. Footpad *papillae* histological structure

Table I – 1. Summary of studies that evaluated effects of protein and energy level and enzyme inclusion on footpad dermatitis

Reference	Treatments	FPD score	Protein level / Dietary density	Enzyme	Summary of results
Bilgili et al., 2006	2 x 2 x 2 factorial arrangement with strain cross (A, B), sex(male, female) and dietary density (low, high)	0 = no lesion 1 = mild lesion (lesion ≤ 7.5 mm) 2 = severe lesion (lesion > 7.5 mm	Starter: CP (%) Low 21.01, High 21.51; ME (kcal/kg) Low 3,109, High 3,193 Grower: CP (%) Low 19.76, High 20.00; ME (kcal/kg) Low 3,158, High 3,226 Finisher: CP (%) 17.08; ME (kcal/kg) 3,248	-	- Interaction effect of strain cross and dietary density on incidence and severity of FPD at 42 d - Low density diets reduced incidence of paw lesions at 35, 42, 49, and 56 d - Males had higher incidence and severity of lesions
Nagaraj et al., 2007a	2 x 2 x 2 factorial arrangement with protein level (high, low), protein source (SBM, SBM + PBPM) and sex (male, female) as main factors	0 = no lesion 1 = mild lesion; crust < 1.5cm in diameter 2 = severe lesion; ulcer > 1.5cm in diameter	Low: SBM; SBM + PBPM — High: SBM; SBM + PBPM (%) Starter: 21.8; 22.3; 24.7; 25.5 Grower: 20.2; 19.9; 22.4; 20.4 Finisher: 16.4; 17.2; 20.2; 18.4 Withdrawal: 18.6; 16.6; 19.6; 17.4	-	- SBM based diets increased FPD at 29 d - No effects were observed at 43 d - At 54 d both high protein and SBM diets increased the incidence and severity of FPD - Males had higher severity scores than females at 54 d
Nagaraj et al., 2007b	2 x 2 x 2 factorial arrangement with protein level (high, low), protein source (SBM, SBM + PBPM) and enzyme inclusion (with, without) as main factors	0 = no lesion 1 = mild lesion; crust < 1.5cm in diameter 2 = severe lesion; ulcer > 1.5cm in diameter	Low: SBM; SBM + PBPM — High: SBM; SBM + PBPM (%) Starter: 21.8; 22.3; 24.7; 25.5 Grower: 20.2; 19.9; 22.4; 20.4 Finisher: 16.4; 17.2; 20.2; 18.4 Withdrawal: 18.6; 16.6; 19.6; 17.4	Allzyme Vegpro ® (0.06 %) - Protease (3.4 million HUT/lb) and Cellulase (20,000 CMCU/lb)	- Protein source effect – all SBM diets higher incidence and severity of FPD - At 57 d mild lesions were affected by protein source enzyme interaction. Enzyme reduce FPD severity in SBM diets
Cengiz et al., 2012	2 x 2 x 2 factorial arrangement with barley inclusion (0, 25%), litter moisture (with, without added water) and enzyme inclusion (0, 0.1%) as main factors	0 = no lesion 1 = mild lesion (lesion ≤ 7.5 mm) 2 = severe lesion (lesion > 7.5 mm	-	Farmazyme BR - M® (0.1%) – xylanase, β-glucanase, cellulose, pectinase, amylase, and mannanase	- No effect of enzyme inclusion were observed on FPD (14, 28, and 42 d) - Wet litter effect – wet litter increased severe and total lesions at 14 d and mild lesions at 28 d.

[1] SBM – Soybean meal
[2] PBPM – Poultry by-product meal
[3] Allzyme Vegpro®- Alltech Biotechnoloy Center, Nicholasville, KY 40356, USA

CHAPTER II

Effects of Eggshell Conductance and Incubation Temperatures on Duck Footpad Development

ABSTRACT

Footpad dermatitis (**FPD**) is a major animal welfare and economical concern. A field trial was conducted to evaluate the effects of eggshell conductance (**G**) and incubation temperature profiles (**TEM**) on duck footpad structure at hatch and 35 d of age, and their influence on FPD incidence. A total of 10,000 Pekin duck eggs were randomly sorted, equally distributed into four groups, and placed into two single stage incubators. Treatments consisted of two G, reduced and normal, and two TEM, elevated and normal, after d 12. Eggshell G was reduced by dipping eggs in wax at 14 d of incubation. At hatch, 6 ducklings from each treatment were weighed and footpad skin samples collected for histological processing. The remaining ducklings were identified and placed in a commercial house. At 35 d, 5 drakes and 5 hens of each treatment combination were weighed and classified for FPD using a three level scale. Additionally, footpads of 7 ducks per treatment combination were sampled. Histological analysis was used to assess thickness and total area of *stratus corneum* (SC), epidermis and dermis and total height and width of footpad *papillae*. For the histological data a CRD with 2x2 factorial arrangement of treatments was used. FPD data were analyzed using cumulative logistic regression to model the log odds of the cumulative probabilities of lesions as a function of the factorial effects of treatments plus the additive sex effect. There were no effect of treatments on BW at hatch and 35 d. High TEM and reduced G increased residual yolk. On footpad measurements, an interaction effect was observed on dermis length and area and total *papillae* height at hatch. At 35 d reduced G ducks had an increased area of epidermis than normal G. Additionally, either elevated TEM or reduced G increased the probability of having FPD. In conclusion G and incubation T have an effect on FPD in

ducks at market age, and this might be related to changes in footpad skin structure during embryo development.

Key words: footpad development, FPD, ducks, welfare

INTRODUCTION

Duck meat is the third most produced poultry meat type in the USA, with an annual estimated production of 75 tons (USDA, 2012). In the US market, ducks are excluded from the Animal Welfare Act (Joy, 2005). In the absence of governmental laws about duck management and welfare, most states consider normal or common farming practices to be the rule on duck production (Joy, 2005). A central parameter of animal welfare is health, since increases in injuries or disease are usually related to poorer welfare (Broom, 2006).

Lesions of the footpads have been widely used in poultry production as a welfare parameter (Shepherd and Fairchild, 2010). This type of lesion is a pathological condition characterized by lesions on the footpads (Nairn and Watson, 1972; Wise, 1978; Martland, 1985; Schulze Kersting, 1996) and ducks have been reported to also be affected (Gibson, 2007; Jones and Dawkins, 2010). These lesions can be observed under a wide range of severity. Initially, discoloration and hyperkeratosis of footpad skin is observed, and in severe stages deep ulceration with extreme swelling of foot (bumblefoot) can occur (Mayne et al., 2005; Jones and Dawkins, 2010). Secondary bacterial infections are common, compromising animal health (Mayne et al., 2005). In FPD conditions, skin structure presents acanthosis with individualization and thickening of keratin layers in the *stratus corneum* (Pass, 1989; Platt et al., 2001). Compared to chickens and turkeys, footpad skin of ducks is especially prone to these lesions because it is weaker (Joy, 2005).

Considering that ducks usually are raised on rough and abrasive surfaces, such as slats and wire mesh, skin structure and resistance to damage are extremely important to avoid injuries (Joy, 2005). Furthermore, when raised on litter, FPD is also likely to occur mainly due to poor litter management (Rosenberg et al., 2005). In duck production, litter management is a key factor since duck excreta has much greater water content than chicken and turkey (Joy, 2005).

Welfare and health of waterfowl are also related to water access and supply (Knierim et al., 2004; Jones et al., 2009; O'Driscoll and Broom, 2011). Access to open water reduces the incidence of footpad and toe lesions (Knierin et al., 2004). O'Driscoll and Broom (2011) also observed that ducks reared using wide-lip bell drinkers tend to have better footpad scores than ducks reared using nipple drinkers. Usually litter characteristics, management, nutrition and genetics are considered to be the most important factors on FPD onset (Mayne, 2005; Mayne et al., 2007a, Shepherd and Fairchild, 2010; De Jong et al., 2012). Although these parameters are common to all individuals, in a flock several degrees of FPD affection are observed. Following this, other parameters that can affect duck structural development, such as incubation, should be evaluated.

Incubation conditions have been reported to influence embryo development and post-hatch performance of turkeys (Christensen et al., 2005) and chickens (Leksrisompong et al., 2007; Lourens et al., 2007; Hulet et al., 2007; Molenaar et al., 2011). The management provided during this period has an impact on organogenesis, metabolic processes, immunity and poultry livability post-hatch. Parameters such as temperature and hypoxia conditions were related to impairment of poultry body structures. High temperature and low oxygen exposure can be detrimental to chicken and turkey embryos,

reducing yolk free BW, and heart, liver and intestinal development (Christensen et al., 2005, Leksrisompong et al., 2007; Lourens et al., 2007). Additionally, Yalçin et al. (2008) reported effects on beak development, observing that these parameters can also affect keratinaceous tissues . Recently, De Jong et al. (2012) observed an effect of hatcheries on FPD incidence in chickens reinforcing a hypothesis that factors that affect embryonic development may be very important for FPD etiology.

There is a lack of information about the effect of prenatal conditions on duck development and health. It is also important to ascertain if incubation conditions have any effect on footpad development affecting FPD incidence. The aim of this study was to investigate the impact of incubation temperatures and hypoxia conditions on duck footpad development.

MATERIAL AND METHODS

Experimental Treatments

A total of 10,000 Pekin duck eggs from a commercial line of Maple Leaf Farms Inc. (Milford, IN, USA) were randomly sorted equally into four groups of 2,500 and incubated in two single stage incubators.

Incubation treatments consisted of two incubation temperature profiles (**TEM**), elevated and normal, and two eggshell conductances (**G**), reduced and normal. Incubation temperature treatments were conducted as shown in Figure II - 1. An incubator was programed to follow the normal incubation temperature profile observed commercially, where the incubator decreases the environmental temperature progressively to guarantee an optimum eggshell temperature. The second incubator was programed to maintain an

environmental temperature of 37.8°C after the 6th d of incubation creating elevated eggshell temperatures as the embryo developed.

At egg setting, 200 eggs of each group were selected and weighed at 0 and 21 d post-setting to calculate moisture loss. On the 14th d of incubation, reduced eggshell conductance was obtained by immersing 1/3 of the egg in warm paraffin to reduce the respiratory surface and create hypoxia conditions. Eggshell conductances for the four treatments were calculated and are presented in Table II - 1. At 24 d, eggs were placed into individual net bags to keep track of egg number, and transferred to hatchers, maintaining the respective temperature treatment profile. At hatch, ducklings were identified according to treatment groups by web punch to be able to identify each treatment combination when housed.

Birds and Husbandry

On this field trial, the duck hatchlings were taken to a commercial farm and placed in 4 pens within a house. The house was curtain sided with nipple drinkers and a plastic wire mesh floor. During the grow-out period all the management and husbandry followed Maple Leaf Farm, Inc. procedures. Feed scheme and composition followed the genetic line specifications.

Data Collection

At hatch, 6 ducklings from each treatment were euthanized, weighed, and sampled for footpads using a 5 mm circular biopsy punch, and residual yolk was determined. At 35 d, at the slaughter plant during unloading of the truck, 7 ducks per treatment combination were randomly selected, euthanized, weighed and the footpads sampled

using a scalpel. Additionally, 5 males and 5 females of each treatment combination were randomly selected and evaluated for FPD. Ducks were classified for FPD using a three level scale (0 to 2). Ducks with no footpad lesions were classified as 0. If a lesion occupied less than 50% of a footpad ducks were classified as 1. Lesions that were severe and greater than 50% of the pad were classified as 2.

Both footpads collected from hatch and 35 d were individually affixed to a tongue depressor to avoid shrinkage, and stored in a 10% formalin solution for 48 h. Afterwards they were transferred to a 70% ethyl alcohol solution for further histological processing. Prior to histological processing, 35 d footpad surface photographs were obtained using a stereoscope microscope with 1.8x zoom to evaluate individual *papillae* surface area. Later on, cross footpad skin sections of 4 - 5 µm were stained with Masson's trichrome technique. Slides were then examined by light microscopy, and photographs of individual *papillae* present in the footpads were taken with zoom of 200x and 40x for hatch and 35 d, respectively.

Morphometric analyses of photos were performed using Image Tool Software (Version 3.0, University of Texas Health Science Center, San Antonio, TX, USA). Measurements assessed thickness and total area of *stratus corneum* (**SC**), epidermis and dermis, and total height and width of papillae. On the footpads of the hatchlings, five *papillae* were used and measured in three different points within the *papillae*, for each parameter. At 35 d, three *papillae* were selected and measured in five different points within the *papillae* for each parameter. For surface area analysis, 10 *papillae* per footpad were randomly selected and measured.

Data Analysis

Performance and histological data were analyzed as completely randomized design in a 2x2 factorial arrangement of treatments, using JMP 10 (SAS Inst. Inc., Cary, NC) software. Before analysis, dermis thickness and SC and dermis area at hatch were transformed to natural logarithms in order to obtain a normal distribution of data. The 35 d SC and dermis length, and the epidermis, dermis and surface area were also transformed to natural logarithms. For the ordinal FPD scores cumulative logistic regression was used to model the log odds of the cumulative probabilities of lesions as a function of the factorial effects of conductance and temperature plus the additive sex effect. Additionally, expected cell counts were computed to compare with the observed cell counts and look for possible lack of fit. For these data the GLIMMIX Procedure of SAS (SAS Inst. Inc., Cary, NC) was used.

RESULTS

BW, residual yolk and footpad skin development at hatch

At hatch neither G nor TEM affected ($P>0.05$) duck yolk free BW (Table II – 2), although, treatments did have an effect ($P<0.01$) on residual yolk. Both stressful conditions, reduced G and elevated TEM, inhibited yolk utilization by the embryos. Hatchlings coming from eggs under hypoxia conditions had higher residual yolk (7.4%) than observed in normal G ducklings (4.0%). Elevated TEM increased residual yolk by 2.4% in comparison with normal TEM.

Regarding histological measurements, only dermis structure was affected ($P<0.001$) by treatments. Both dermis length and area were affected ($P<0.001$) by treatment interaction. Thickness and area of dermis were maximized under normal TEM and G,

although statistically normal and stressful incubation conditions induce the same dermis development. Nevertheless, it seems that normal TEM produce a thicker epidermis.

On overall *papillae* structure, only height was affected ($P<0.001$) by a treatment interaction. The results were similar to those observed on dermis which indicates that at hatch *papillae* height was manly affected by dermis thickness. No effects ($P<0.05$) were observed on *papillae* width.

BW and skin development at 35 d

Market age duck BW was not affected ($P>0.05$) by treatments. Histological analyses of footpads indicated an effect ($P<0.01$) of treatments on epidermis development. Even though no significant effect ($P>0.05$) was observed on epidermis thickness, total area was diminished under elevated TEM conditions. No other skin layer was affected ($P>0.05$) by treatments used.

The general *papillae* structure evaluation indicated a TEM effect ($P=0.087$) was observed on *papillae* width. Ducks incubated with normal TEM had a thicker *papillae* basis than the ones incubated with elevated TEM. Additionally, when measuring *papillae* surface area, elevated incubation temperatures increased ($P<0.05$) individual *papillae* area of footpads. No effects ($P>0.05$) were observed on *papillae* height.

On the FPD score evaluation independent effects ($P=0.078$) of temperature and conductance were observed. Ducks exposed to high incubation temperatures and hypoxia conditions had a higher probability to have scores 1 and 2 of footpad lesions. Alternatively, ducks coming from eggs with normal eggshell conductance and incubation temperatures had a higher probability to have no lesions on the footpads. No differences

(P>0.05) of FPD scores were observed between sexes. The simple model used in this analysis had a good fit (X^2=40, df =35).

DISCUSSION

Based on the results observed at hatch, stressful conditions during incubation impacted yolk utilization by the embryo. Residual yolk increased when eggs were incubated under hypoxia conditions. Metcalfe et al. (1984) argued that when part of the eggshell is covered reducing oxygen concentration during incubation, a retarded growth of chicken embryos is observed. Christensen et al., (2005) and Lourens et al. (2007) found the same trends of hypoxia conditions on BW without yolk in turkeys and chickens respectively. Even though in this field trial no effect of G was observed on free yolk BW, yolk utilization was affected. Reducing eggshell surface decreased yolk mobilization by the embryo at hatch.

Elevated TEM has been reported to affect embryo development (Christensen et al., 2005; Leksrisompong et al., 2007; Lourens et al., 2007). Again no effects were observed on yolk free BW but residual yolk was increased under elevated TEM. Furthermore, Hulet et al. (2007) related decrease performance of broiler chickens at slaughter age when incubated with TEM (39.7°C eggshell temperature). The same findings were reported by Molenaar et al. (2011) when incubating eggs at 38.9°C of eggshell temperature. Despite these reports, no differences in BW of ducks at market were detected among the small sample evaluated in this experiment.

The footpad structure analysis, showed only dermis thickness and area were affected by treatment interaction at hatch. Normal G and TEM ducklings had the thicker and biggest dermis area, though they were statistically equal to the ones coming from reduced

G and elevated TEM. Dermis development of eggs incubated with normal TEM was negatively affected by hypoxia conditions. Dermis layer is especially important since is the place where skin collagen is located, being this way directly related with skin strength (Ramshaw et al., 1986; Christensen et al.,1994).

When evaluating skin development at 35 d, it was observed that differences in dermis development were no longer observed, but that the epidermis was affected by TEM. One of the typical histological findings of FPD is hyperplasia of epidermis with thickening of this layer (Pass, 1989). In the present study no effects were observed on thickness of epidermis but normal TEM increased the total area of epidermis. At 35 d the *papillae* surface area was increased by elevated TEM, but the width of the basis of *papillae* was reduced by this incubation temperature profile. Therefore the width at the basis of *papillae* was wider in ducks incubated with normal TEM, though when evaluating the top, normal TEM decreased the width of the *papillae* (Figure II – 2).

Our hypothesis was that stressful conditions would negatively affect skin development and strength, influencing FPD incidence. Kafri et al. (1986) evaluated breast, thigh, back and side skin strength of broilers and state that skin strength is directly related to thickness of epidermis and dermis. We observed that the probability of FPD development and severity increases when reduced G and elevated TEM are used. Interestingly, at hatch birds of the treatment combination of normal eggshell temperatures with normal G had a thicker and larger dermis area; therefore FPD can be related to dermis structure at hatch. Conversely, this treatment combination induced the same changes of dermis structure as the treatment combination of elevated TEM and reduced eggshell conductance. At 35 d, the increased area of epidermis on normal TEM ducks indicates that this layer might be related to FPD since the probability of not having FPD

was higher in ducks under this condition. Furthermore, the changes observed on *papillae* width and surface area by elevated TEM can also be correlated with severity FPD. Wider top of *papillae* with thinner basis may be an indicator of higher susceptibility to FPD.

In this study, it should be considered that the sample size used was probably not enough to detect clear patterns of stressful incubation conditions on footpad skin development and FPD causation. Nevertheless, it was pertinent that treatments did have an effect on embryo yolk utilization and impacted FPD severity. In conclusion, incubation conditions may be an explanation for differences in FPD incidence between flocks or between individuals within the same flock.

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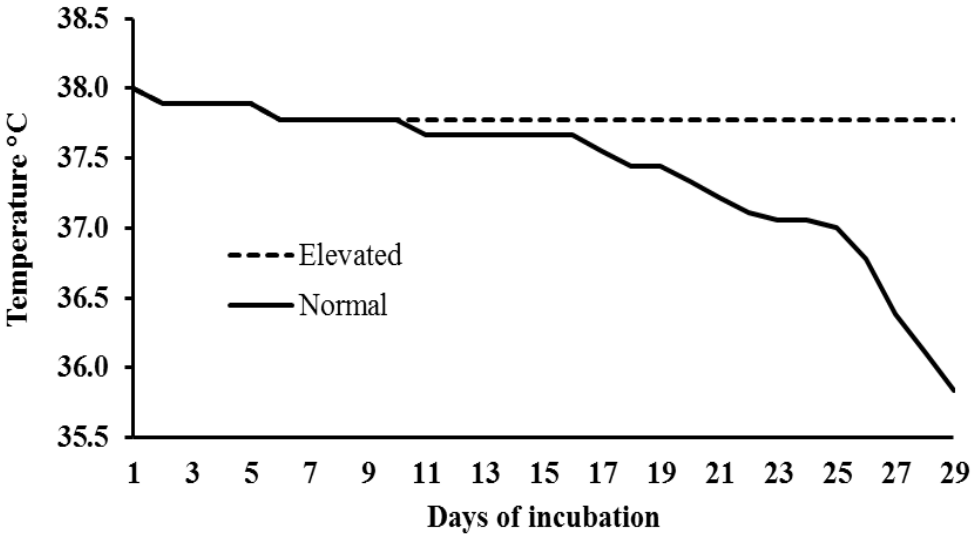

Figure II - 1. Incubation temperature profiles for each treatment.

Table II - 1. Moisture loss percentage and eggshell conductance for each treatment combination

Treatment		Moisture loss mean (%)	Conductance[2] (mg/torr/day)
Incubation temperature	Eggshell conductance		
Normal	Normal	11.70 ± 0.13[a]	547 ± 6[a]
Normal	Reduced	10.03 ± 0.13[b]	469 ± 6[b]
Elevated	Normal	11.65 ± 0.13[a]	542 ± 6[a]
Elevated	Reduced	10.11 ± 0.13[b]	470 ± 6[b]

[1]Values are means ± SE of 200 eggs per treatment combination

[2]Conductance = $\frac{\text{Initial weight} - \text{final weight}}{\text{partial pressure inside the egg} - \text{partial pressure outside the egg}}$

[a,b] Means in a column not sharing a common superscript are significantly different ($P<0.05$) by Tukey's test

Table II - 2. Effect of eggshell conductance and incubation temperature profiles on duck BW (BW) at hatch and 35 d and residual yolk at hatch

Eggshell conductance	Incubation temperature	Hatch		35 d
		BW (g)[2]	Residual Yolk (%)	BW (g)
Normal		48	4.5[b]	3224
Reduced		49	6.9[a]	3193
	Normal	49	4.0[b]	3238
	Elevated	48	7.4[a]	3179
Normal	Normal	49	2.9	3237
Normal	Elevated	47	5.1	3211
Reduced	Normal	50	6.1	3240
Reduced	Elevated	48	8.7	3147
Pooled SEM		2	1.6	239
Source of variation		---------- *P*–values ----------		
Eggshell conductance		0.221	<0.001	0.435
Incubation temperature		0.164	0.004	0.687
Eggshell conductance x Incubation temperature		0.786	0.825	0.663

[1]Values are means of 6 ducks for hatch, and 7 ducks for 35 d
[2]BW without yolk
[a-b]Means in a column not sharing a common superscript are significantly different ($P<0.05$) by Student's t test

Table II - 3. Effect of eggshell conductance and incubation temperature profiles on footpad skin *stratus corneum*, epidermis and dermis thickness at hatch and 35 d

Eggshell conductance	Incubation temperature	*Stratus corneum*		Epidermis		Dermis	
		Hatch	35 d	Hatch	35 d	Hatch	35 d
		---------- µm ----------					
Normal		30	178	48	170	187[a]	513
Reduced		26	181	53	149	170[b]	433
	Normal	28	171	48	172	176	450
	Elevated	27	188	53	147	181	496
Normal	Normal	32	160	44	179	208[a]	493
Normal	Elevated	28	197	52	161	165[bc]	533
Reduced	Normal	25	183	52	166	153[c]	407
Reduced	Elevated	27	180	54	133	186[ab]	459
Pooled SEM		5	56	11	41	17	124
Source of variation		---------- *P*–values ----------					
Eggshell conductance		0.118	0.951	0.369	0.231	0.056	0.116
Incubation temperature		0.723	0.413	0.346	0.139	0.615	0.397
Eggshell conductance x Incubation temperature		0.213	0.435	0.617	0.662	<0.001	0.821

[1]Values are means of 6 ducks for hatch, and 7 ducks for 35 d
[a-c]Means in a column not sharing a common superscript are significantly different ($P<0.05$) by Student's t or Tukey's test

Table II - 4. Effect of eggshell conductance and incubation temperature profiles on footpad skin *stratus corneum*, epidermis and dermis areas at hatch and 35 d

Eggshell conductance	Incubation temperature	*Stratus corneum*		Epidermis		Dermis	
		Hatch	35 d	Hatch	35 d	Hatch	35 d
		---------- **µm^2 × 10^3**----------					
Normal		12.5	216.2	16.2	184.2	52.9	466.6
Reduced		12.2	214.5	18.2	182.0	50.0	489.8
	Normal	13.5	216.8	15.8	210.0[a]	51.2	495.1
	Elevated	11.2	213.8	18.6	156.3[b]	51.6	461.4
Normal	Normal	14.2	206.0	15.1	198.7	60.1[a]	472.8
Normal	Elevated	10.8	226.4	17.3	169.8	45.6[bc]	460.5
Reduced	Normal	12.9	227.7	16.5	221.2	42.4[c]	517.3
Reduced	Elevated	11.5	201.2	20.0	142.8	57.6[ab]	462.3
Pooled SEM		5.0	52.8	4.4	38.2	9.3	192.2
Source of variation		---------- *P*–values ----------					
Eggshell conductance		0.656	0.936	0.349	0.587	0.528	0.837
Incubation temperature		0.330	0.887	0.196	0.002	0.936	0.655
Eggshell conductance x Incubation temperature		0.523	0.280	0.754	0.074	<0.001	0.912

[1]Values are means of 6 ducks for hatch, and 7 ducks for 35 d

[a-c]Means in a column not sharing a common superscript are significantly different (P<0.05) by Student's t or Tukey's test

Table II - 5. Effect of eggshell conductance and incubation temperature profiles on footpad *papillae* height width at hatch and 35 d and surface area at 35 d.

Eggshell conductance	Incubation temperature	Height		Width		Surface area
		Hatch	35 d	Hatch	35 d	35 d
		---------- µm ----------				mm^2
Normal		265	846	320	1032	1.07
Reduced		248	757	323	1107	1.04
	Normal	256	792	337	1106	1.00[b]
	Elevated	257	811	306	1033	1.12[a]
Normal	Normal	284[a]	842	314	1064	0.99
Normal	Elevated	246[bc]	850	326	1000	1.16
Reduced	Normal	229[c]	741	299	1149	1.01
Reduced	Elevated	267[ab]	772	347	1065	1.08
Pooled SEM		25	137	39	102	0.22
Source of variation			---------- *P*–values ----------			
Eggshell conductance		0.188	0.121	0.884	0.080	0.7692
Incubation temperature		0.982	0.732	0.124	0.087	0.0267
Eggshell conductance x Incubation temperature		<0.001	0.835	0.359	0.811	0.3632

[1]Values are means of 6 ducks for hatch, and 7 ducks for 35 d
[a-c]Means in a column not sharing a common superscript are significantly different ($P<0.05$) by Student's t or Tukey's test

Table II - 6. Effect of eggshell conductance and incubation temperature profiles on incidence footpad dermatitis at 35 d

Conductance	Temperature	Footpad dermatitis score		
		Probability		
		0	1	2
Normal	Normal	0.71	0.25	0.04
	Elevated	0.24	0.54	0.22
Reduced	Normal	0.81	0.16	0.03
	Elevated	0.71	0.25	0.04
Source of variation		--------- *P*–values ---------		
Conductance		0.078		
Temperature		0.078		
Conductance x Temperature		0.276		
Sex		0.510		

[1]Score 0 = no lesion present
[2]Score 1 = lesion < half of the footpad
[3]Score 2 = lesion > half of the footpad

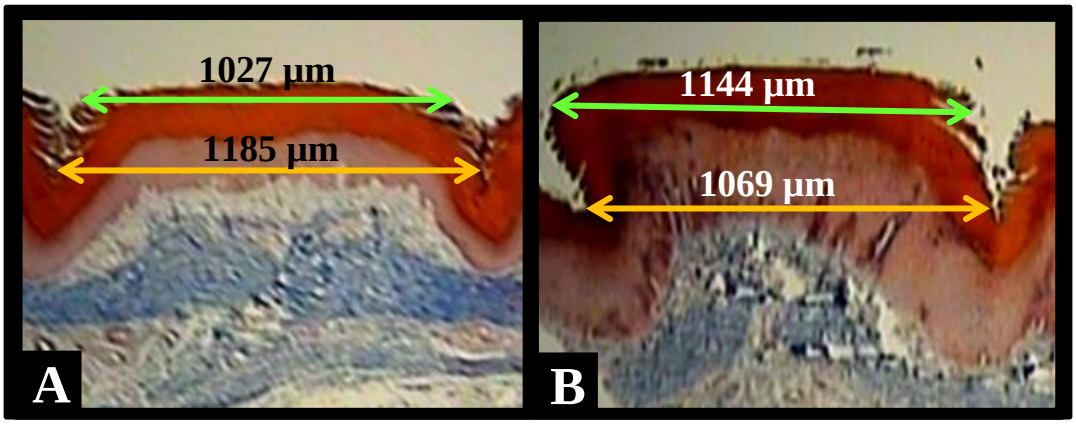

Figure II - 2. Differences in width on the top and basis of footpad skin *papillae* between ducks incubated with normal (A) and elevated (B) incubation temperatures.

CHAPTER III

Breeder Feeding Restriction Programs and Incubation Temperatures Effect on Progeny Footpad Development

ABSTRACT

Footpad dermatitis initiates early in life, and there is evidence of individual susceptibility. An experiment was conducted to evaluate the carryover effects of breeder feed restriction programs and incubation temperatures (**TEM**) on progeny footpad development at hatch, 7 and 22 d. Cobb 500 fast feathering breeders were subjected to two dietary feed restriction programs during rearing, skip-a-day (**SAD**) and every-day feeding (**EDF**). At 60 wk of age, eggs from each group were collected and incubated according to two TEM, standard (**S**) eggshell temperature 38.1^{o}C and early-low late-high (**LH**). This second profile had low (36.9^{o}C) eggshell temperature for the first 3 d, and standard temperature until the last 3 d when eggs endured elevated (38.9 ^{o}C) eggshell temperature. At hatch, 15 chicks from each treatment combination were sampled to obtain footpads for histological analysis. Seventy two chicks per treatment were placed in 48 cages (6/cage), and raised to 22 d. At 7 and 22 d, one and two chickens, respectively, were sampled for footpads. The BW and group feed intake were recorded to obtain BW gain, FCR at 7 and 21 d. Histological analysis assessed thickness and total area of *stratus corneum* (**SC**), epidermis and dermis and total *papillae* height. Data were analyzed as RCBD in a 2x2 factorial arrangement of treatments. There was a negative effect of LH TEM on performance at both ages. An interaction effect on SC area and *papillae* height was observed at hatch. Additionally SAD treatment increased thickness and area of footpad dermis. At 7 d, the SC parameters of the SAD progeny were increased. Epidermis thickness was affected by treatment interaction. Furthermore LH TEM decreased epidermis thickness and dermis area. At 22 d, interaction effects were observed in thickness and area of SC and epidermis. Incubation S TEM increased thickness and area

of dermis. It was concluded that breeder feed restriction programs and incubation TEM profiles may have carryover effect on histomorphological traits of footpads.

Key words: footpad development, broilers, incubation, breeder, feed restriction

INTRODUCTION

In today's commercial broiler production, lesions on footpads are a common finding leading to economical and production losses and raising concern about animal welfare (Berg, 1998; Breuer et al., 2006). The skin of footpads is considered a specialized epidermal type with reticular scales that protect birds against environmental hazards (Michel, 1992). Footpad dermatitis (**FPD**) condition is most likely to have multifactorial origin (Shepherd and Fairchild, 2010; De Jong et al., 2012). It has been shown that wet litter, together with nutrition are the major causative agents of FPD (Shepherd and Fairchild, 2010). Skin strength, bird weight, sex and breed may also be related to the development of these lesions (Mayne, 2005).

Carry over effects of parents to progeny and the egg incubation conditions play an important role on development of several structures of broilers during embryogenesis (Triyuwanta et al., 1990; 1992; Breuer et al., 2006; Romero, 2008; Eusebio-Balcazar, 2010). Triyuwanta et al. (1990; 1992) showed that breeder management and feed scheme impact the progeny. Hens subjected to feed restriction programs had offspring with lower tibial bone breaking force, and BW at hatch and market age were lower. Furthermore, Breuer et al. (2006) compared the footpad condition of turkey progeny of two different age hen flocks (4 and 20 wk of production), and observed a poorer footpad health condition of the offspring from the older breeders.

Incubation environment also impacts embryo development and affects progeny performance and health. Temperatures below or above the optimum level may impact embryonic growth rate and development. Recently, Yalçin et al. (2008) showed that higher incubation temperatures between 10 and 18 d of incubation increased BW at hatch, but decreased liver and heart rates. Several other researchers also showed that incubation temperatures affect embryo yolk utilization and organogenesis (Leksrisompong et al., 2007; Oviedo-Rondón et al., 2009; Molenaar et al., 2011). Other consequences of improper incubation temperatures are the changes in hormonal and metabolic regulations that may alter development (Moraes et al., 2004; Yahav et al., 2004; Piestun et al., 2009). Reduction in concentration of plasma thyroid hormones are observed when the eggs are exposed to high temperatures during incubation (Yahav et al., 2004; Piestun et al., 2009). Furthermore, Moraes et al. (2004), demonstrated that embryo blood corticosterone during incubation may be increased due to heat stress situations. These same hormonal changes have also been reported to affect skin thickness in humans and animal diseases. Hyperadrenocorticism is strongly related to thinner skin in Cushing disease in mammals (Sheppard et al., 1966; Safer et al., 2003). Additionally, chicken scale morphogenesis has been reported to be negatively affected by cortisone and hydrocortisone (Moscona and Karnofsky, 1960; Stuart et al., 1972; Fisher, 1984).

All these prehatch parameters may also affect the development of footpad structures and reticulate scales that cover the plantar surface of feet, and may also be part of predisposing factors that increase or decrease the incidence of FPD. In fact, epidermal-dermal interactions during the development of avian integument have an important role on the modulation of morphogenesis of skin and its appendages; feathers, spurs, scutate scales, and reticulate scales are impacted by these interactions (Sawyer et al., 1984).

One of the major histomorphological alterations observed in FPD is hyperkeratosis (Shepherd and Fairchild, 2010). Platt et al. (2001) reported a hyperkeratosis of footpads plus separation of keratin layers in foot lesions on turkey poults at 6 wk. Other authors also concluded that, in severe ulcerated lesions, *stratus corneum* (**SC**) shows evident defects as thickening and formation of 'horned pegs' (Martland, 1984; Whitehead, 1990). Additionally, in a study conducted by Kafri et al. (1986) to evaluate breast, thigh, back and side skin strength, the authors found that this parameter is inversely related to hypodermis thickness. The authors also reported that thicker epidermis and dermis lead to higher skin strength.

Since foot health is an indicator of animal welfare and a main source of revenues in the industry, we studied the major histomorphological alterations on the three layers of broiler footpads – SC, epidermis and dermis during embryo development. In the present experiment, footpad skin of chickens was analyzed to evaluate the carryover effects of two different breeder feed restriction programs and incubation temperatures on progeny footpad development at hatch, 7 and 22 d of age.

MATERIAL AND METHODS

Breeder Treatments

Two groups of six hundred Cobb 500 fast feathering d old breeders were randomly divided and housed in six pens. The two groups were subjected to two dietary feed restriction programs, skip-a-day (**SAD**) and every-day feeding (**EDF**), which started at 2 wk of age and ended at 20 wk at the time of the transfer to the laying pens. In the EDF feed scheme, breeders were fed once every 24 h. The SAD consisted of a feed allotment of every other d, having the quantity fed equal to the one given to the EDF treatments

during two d. In both treatments, chickens were fed by an automatic chain feeder. At the 20th wk of age, breeders from both treatments were selected so the BW profile was similar across the pens. Afterwards they were transferred to laying pens to obtain a total of 46 females to 4 roosters. During all the laying period, breeders were fed once a d at 6:30 in the morning. At 60 wk of age, a total of 800 eggs laid during four consecutive d were collected and stored at 19°C before being set to incubate.

Incubation Treatments

Two groups of 400 eggs per breeder treatment were identified, weighed and set randomly among 6 incubator trays (~160 eggs per tray) with all treatments represented uniformly in each tray. These 6 trays were randomly placed into two Jamesway 252B machines (Butler Manufacturing Co., Ft. Atkinson, WI) for incubation and then transferred to two hatchers. Each machine represented one of the two incubation temperature profile (**TEM**) treatments using a relative humidity of 55% and turning every h the eggs a 45° angle from vertical. The first machine was considered the standard (**S**) treatment and was operated to obtain normal eggshell temperatures (37.5 - 38°C) during the first 18.5 d of incubation. The second machine was considered the low-high (**LH**) TEM. This treatment intended to simulate abnormal TEM observed commercially where embryos are exposed to low TEM during the first d of incubation and high TEM during the last d. For the first 4 d, the incubator was set to obtain eggshell temperatures of 36.9°C. After this period, eggshell temperatures were raised gradually to standard temperatures (37.5 – 38.1°C) until the egg transfer to the hatchers.

At egg transfer, 18.5 d after setting, eggs were weighed and transferred to individual hatching bags to allow tracking of the chick with an egg. Eggs coming from the S

treatment were transferred, and then hatched in a machine programed to obtain eggshell temperatures of 37.5°C. The other hatcher received eggs coming from the low TEM incubator and was operated to achieve eggshell temperatures of 38.9°C.

At hatch, 15 chicks from each treatment combination were weighed and sampled to obtain skin footpad using a circular biopsy punch with a 5 mm diameter, on both feet. Tissues were place on a dry paper to avoid shrinkage and stored in a 10% formalin solution for 48 h. After fixation tissues were transferred to a 70% alcohol solution for further histological processing.

Birds and Husbandry

At hatch, seventy two chicks per treatment were randomly selected and identified with a neck tag. Afterwards, they were placed in 48 Petersime cages divided in three independent blocks inside the room, with 6 broilers per cage, and raised up to 22 d. Birds were fed a common corn soybean based diet *ad libitum* (Table III-1.) Room temperature and lighting program were checked daily and followed the commercial practices.

Data Collection

Chickens and feed were weighed at hatch, 7 d and 21 d for performance analysis. Group feed intake and individual BW gain were calculated for the 0 to 7 and 0 to 21 d periods. Mortality was recorded daily to calculate and adjusted feed conversion ratio. At hatch, 15 chicks from each treatment combination were weighed and sampled to obtain skin footpad using a circular biopsy punch with 5 mm of diameter, on both feet. Tissues were fixed in a dry paper to avoid shrinkage and stored in a 10% formalin solution for 48 h. After fixation the tissues were transferred to a 70% alcohol solution for further

histological processing. At 7 and 22 d, one and two birds respectively, were randomly selected per cage and killed by cervical dislocation. BW were recorded and right footpad samples collected as previously described. At 22 d, the left footpad was collected using a scalpel for *papillae* surface area evaluation. The right foot footpad tissues for the three ages were processed for histology, cut in cross sections of 3 – 5 mm of thickness, and stained using the Masson's trichrome dye.

Photographs of *papillae* present in the footpads were taken using an optical microscope with a camera attached. The magnification of 200x, 100x, and 40x for hatch, 7 d and 22 d was used respectively. Each photo was histologically analyzed using Image Tool Software (Version 3.0, University of Texas Health Science Center, San Antonio, TX, USA). Thickness and total area of SC, epidermis and dermis and total height and width of *papillae* were assessed (Figure III - 1). For the hatch and 7 d samples five papillae per footpad were used and measured in three different points, within the papillae, for each parameter. At 22 d three papillae were selected and measured in five different points, within the papillae for each parameter. Left footpads from hatch and 22 d were used to assess papillae surface area at both ages and papillae number on a 50 mm^2 area at 22 d, using a stereoscopic microscope. Zoom of 2.3x was used for hatch and 1.8x for 22 d.

Data Analysis

Data were analyzed as randomized complete block design in a 2x2 factorial arrangement of treatments. Before analysis, some parameters had to be transformed to natural logarithms in order to obtain normality. Epidermis length, epidermis area and papillae surface area were transformed at hatch. At 7 d, SC, and epidermis length and area

and *papillae* height had to be transformed. At 22 d, epidermis length, and SC, epidermis, and dermis area were also transformed. Also at 22 d, cage block was considered as random effect. All data were analyzed with JMP 9 (SAS Inst. Inc., Cary, NC) software.

RESULTS

Performance

The yolk usage evaluation demonstrated ($P<0.05$) that chickens incubated with LH TEM had higher (16.0%) residual yolk values than the ones incubated with S TEM (14.9%). Based on performance results (Table III – 2), there were neither significant ($P>0.05$) carryover effects of breeder feed restriction programs nor incubation treatments on chicken BW at hatch, although a main effect ($P<0.05$) of incubation treatments was observed on 7 and 21 d BW. Chickens coming from eggs incubated at S TEM were heavier at these ages. Similar effects ($P<0.05$) of S TEM were observed on BW gain and feed intake for 0 to 7 d period and for all the grow out. Optimum feed conversion ratio at 7 and 21 d were obtained ($P<0.05$) using S TEM. No effects ($P>0.05$) of breeder nutrition were observed on feed intake or FCR. No significant effects ($P>0.05$) of treatments were observed on group uniformity.

Footpad Development at Hatch

Based on the histological analysis of the footpads (Table III – 4) at hatch, there were no treatment ($P>0.05$) effect on SC and epidermis length, although a close to significant effect ($P=0.08$) was observed on the SC thickness, with chickens coming from S incubation treatments having a thicker SC layer. Dermis length was affected ($P<0.05$) by the breeder feed restriction program. The SAD program caused an enlargement of this layer. When evaluating the total area of the three different layers treatments had effect on

SC and dermis (Table III – 5). A treatment interaction ($P<0.01$) was observed on SC area. Chicks subjected to LH treatments and coming from SAD breeders had higher areas. Similar to dermis length, evaluation of the dermis area was impacted positively ($P<0.05$) by breeders SAD diet program. The SAD program also increased ($P<0.05$) total papillae height. No differences ($P>0.05$) between treatments were obtained on papillae width and surface area (Table III – 6).

Footpad Development at 7d

The three layers of footpad skin *papillae* were affected by the treatments (Table III – 4). The SC thickness and area were affected ($P<0.05$) by breeder feed restriction program. Progeny coming from SAD breeders had thicker and an increased area of SC than EDF progeny (Table III – 5). Epidermis thickness was affected ($P<0.05$) by treatment interaction. When eggs were incubated with LH profile, broilers coming from SAD feed restriction program had thicker epidermis than the EDF ones. Although, when evaluating total epidermis area only a main effect ($P<0.05$) of temperature was observed. The LH TEM decreased epidermis area. This same effect ($P<0.05$) of incubation TEM was observed on dermis thickness. No effects ($P>0.05$) of treatments were observed on *papillae* height and width (Table III – 6).

Footpad Development at 22d

At 22 d, both SC length and area were affected ($P<0.05$) by treatment interaction (Table III – 4; Table III – 5). Chickens coming from breeders EDF and subjected to S TEM had higher SC length and area values. Thicker epidermis and increased area were also obtained ($P<0.001$) when chickens came from EDF and S TEM or SAD and LH

TEM. Dermis was thicker (P>0.01) in chickens incubated with S TEM; however, no effect (P>0.05) was observed on dermis area.

DISCUSSION

It has been recognized that incubation affects embryo development and impacts yolk absorption (Christensen et al., 2005; Leksrisompong et al., 2007; Lourens et al. 2007; Hulet et al., 2007) and in the present experiment it was observed that LH TEM reduced yolk usage by the embryo. Also, carry over effects of breeder management have been shown to affect progeny (Triyuwanta et al., 1990; 1992; Breuer et al., 2006). Triyuwanta et al. (1992) reported that severe feed restriction during the production phase of broiler dwarf breeders affected progeny BW at hatch and 40 d of life being especially evident on the progeny of ultra-light breeders. Furthermore, severe feed restriction decreased abdominal fat content of female progeny and decreased bone stiffness at hatch and 40 d. Despite these reports, in the present experiment no effects were observed on BW at hatch and live performance of chickens was not affected by the breeder feed restriction programs. This discrepancy of results might be explained by the different time periods when the breeders were fed restricted. Nevertheless, at 7 and 22 d, incubation treatments impacted chicken development. Hulet et al. (2007) reported an effect of hatcher temperature on chicken performance. When eggs were exposed to high temperatures (39.7°C eggshell) in the hatchers, chickens presented higher BW at hatch than chickens hatched under middle and low temperatures. On the other hand, the BW at 21, 35 and 40 d of age was higher in the middle temperature chickens (38.6°C eggshell temperature). Also, the authors observed poor FCR in the high temperature chickens in comparison with the middle and low ones (37.5°C eggshell temperature). Similar effects on BW gain

and FCR were observed on our experiment when comparing with this last trial. Overall, LH TEM negatively impacted BW gain and FCR both at 7 and 21 d. Chickens incubated with LH TEM were on average 39g lighter than the chickens incubated with S TEM.

Breuer et al. (2005) observed that when comparing footpads of poults coming from two different parent stock ages (4 vs. 20 wk of production) the progeny of the older breeders had signs of FPD earlier than the progeny of the younger breeders. Additionally, when evaluating FPD lesions at the 3rd wk of age, the progeny of older breeders had an incidence of 80% while in the progeny of the younger breeders the incidence was 27%. Therefore, there is an evidence of parent stock effect on FPD etiology. Footpad skin layers were affected by the treatments used. The interactions observed on SC and epidermis length at 22 d suggested that TEM and breeder feed restriction programs may be related to FPD susceptibility. It is described that, in footpad lesions, proliferation of SC and hyperplasia of epidermis are present (Pass, 1989). These morphological alterations usually also increase normal *papillae* size. In the present experiment it was observed that birds coming from an EDF parent stock did not have differences in SC area between the two incubation treatments. On the other hand, the progeny from SAD when exposed to LH TEM had larger SC area when compared to S TEM. Therefore, feed restriction of breeders, when combined with stressful conditions during incubation, increased the footpad SC of the chicken early in life. When evaluating the evolution of *papillae* development during the grow-out period, it was observed that at 22 d, the thinnest and smallest areas of SC were observed in the SAD - S treatment combination. Conversely, epidermis measurements demonstrated the same effect as observed at hatch on SC. Interestingly, the theoretical less (EDF – S) and highest (SAD – LH) stress conditions had the highest epidermal growth. Another noteworthy observation is that

progeny of EDF did not show any difference between TEM, while the SAD progeny were affected by the TEM. The LH TEM strongly increased the thickness and the area of epidermis of these chickens, demonstrating that improper incubation temperatures are more deleterious on progeny of breeders that were feed restricted.

Another parameter that may explain FPD triggering is skin strength. Skin strength is mainly related to the collagenous dermal layer of the skin (Ramshaw et al., 1986; Christensen et al., 1994). Also, Christensen et al. (1994) argue that dermis thickness is related positively with skin strength, although Kafri et al. (1986) reported that skin strength relationship with dermis and epidermis thickness might be dependent on skin localization within the body. Taking into account these findings, it is possible that FPD predisposition may also be related with dermis thickness and collagen content of footpad skin. In the present study, we observed that at hatch, SAD progeny had an increased dermis area compared to EDF, although this effect was not promulgated and observed at 7 and 22 d. During the grow-out period a main effect of incubation treatments was observed on dermis thickness and area. Chickens incubated with S TEM had thicker and an increased dermis area at 7 d and a thicker dermis at 22 d. Therefore, incubation conditions impacted dermis development which may be associated with less collagen content of footpad skin.

Using the pairwise correlations (Table III – 6) there was a correlation between BW and dermis area (r = 0.30). This is a weak correlation, but nevertheless it should be regarded as a confounding factor. Finally, based on *papillae* height and width differences between treatments indicated that the overall *papillae* structure was affected by breeder nutrition and incubation conditions. On *papillae* height analysis, care should be taken when interpreting the data presented here in, since it was considered *papillae* height from

the basis of the dermis to the top of SC. Therefore the *papillae* growth observed by Pass (1989) on FPD are mainly related with epidermal layer thickening but in the analysis conducted in this study are influenced by the three layers. At hatch, progeny of SAD had longer *papillae* when incubated with LH than EDF ones. Therefore that footpad skin structure of progeny from SAD breeders is more influenced by TEM than EDF. At 7 and 22 d no carry over effect of breeders was observed on *papillae* height, although chickens exposed to S incubation conditions had taller *papillae* than the LH. This is most likely due to the effect observed on the thickening of the dermis. Also at 22 d a weak correlation between *papillae* height and BW was observed.

Even though no effects were observed on *papillae* width, individual surface of *papillae* was affected by treatments. Again, SAD progeny was differently affected by the incubation treatment. The LH profile increased the individual *papillae* area. Considering that the enlargement of *papillae* with formation of horned pegs is a sign of FPD (Pass, 1989), it can be hypothesized that bigger *papillae* surface area may be precursor sign of FPD. However, when evaluating the *papillae* slides, no keratinaceous abnormality between the treatments was observed.

On pairwise correlation analysis a weak correlation was observed between BW, and dermis area and *papilla* height. One parameter that may bias our results was the chicken sex, since chickens were not sexed. Sexual dimorphism has been reported on FPD condition, but the affection between sexes is not consistent (Bilgili et al., 2006; Nagaraj et al., 2007a,b).

In conclusion, S TEM enhanced chicken performance and increased dermis development. Breeder feed restriction program did not impact chicken performance of progeny. The breeder SAD feed scheme demonstrated that the footpad was more

susceptible to improper development under inadequate incubation temperatures. Nevertheless, it was demonstrated that incubation conditions and parent stock management both impact footpad development, although further research is needed to understand if these morphological changes correspond to different FPD incidence and severity under commercial conditions.

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Table III-1. Composition of starter broiler diet (%) and formulated nutrient contents

Ingredients	Starter basal 1-22 d
	%
Corn	49.95
Soybean meal, 48%	34.42
Distillers dried grains with solubles	6.00
Poultry fat	4.40
Salt (NaCl)	0.42
Limestone	1.52
Dicalcium phosphate, 18.5%	1.11
DL-methionine, 99%	0.27
L-lysine-HCl, 78,8%	0.15
Choline chloride, 60%	0.20
Sodium bicarbonate	0.14
L-threonine, 98%	0.04
Coccidiostat[1]	0.05
Mineral premix[2]	0.20
Vitamin premix[3]	0.10
Phytase[4]	0.02
Filler[5]	1.00
Total	100.00
Nutrient composition	
ME, kcal/kg	3,050
CP, %	22.81
Calcium, %	0.98
Total phosphorus, %	0.64
Available phosphorus, %	0.38
Digestible. lysine, %	1.20
Digestible total sulfur amino acids, %	0.87
Digestible threonine, %	0.77
Digestible tryptophan, %	0.24
Sodium, %	0.24
Potassium, %	0.96
Chloride, %	0.31
Dietary electrolyte balance, mEq/100 g	268

[1]Coban 90® 45 (Monesin Sodium), Elanco Animal Health, Greenfield, IN

[2]The trace mineral premix supplied the following per kilogram of diet: 120 ppm of Zn as Zinc Sulfate, 10 ppm of Cu as Copper Sulfate, 120 ppm of Mn as Manganese Sulfate, 80 ppm of Fe as Iron Sulfate, 2.5 ppm of I as Calcium Iodate, 1 ppm Co as Cobalt Sulfate.

[3]Vitamins from premix provided per kilogram of premix: vitamin A, 18,739,292 IU; vitamin D_3, 6,613,868 IU; vitamin E, 66,139 IU; vitamin B_{12}, 33 mg; riboflavin, 22,046 mg; niacin, 88,185 mg; d-pantothenic acid, 30,865 mg; menadione, 3,968 mg; folic acid, 2,646 mg; vitamin B_6, 7,716 mg; thiamine, 5,512 mg; biotin, 176 mg.

[4]Ronozyme® P CT at 185 g/ton to provide 930 FYT (DSM Nutritional Products, Parsippany, NJ).

[5]Celite, Celite Corp., Lompoc, CA

Table III – 2. Effect of feed restriction programs and incubation temperature profiles on chicken progeny BW and BW gain (BWG), of 60-wk-old Cobb 500 broiler breeders

Breeder feed restriction	Incubation temperature	BW (g)			BW gain (g)	
		0 d	7 d	21 d	0-7 d	0-21 d
EDF		52.60	192	939	140	886
SAD		52.24	195	961	143	908
	Standard	52.45	199[a]	969[a]	146[a]	917[a]
	Low - high	52.41	189[b]	930[b]	137[b]	878[b]
EDF	Standard	52.70	197	958	145	905
	Low - high	52.52	188	920	135	867
SAD	Standard	52.20	200	980	148	928
	Low - high	52.30	190	941	138	889
Pooled SEM		1.34	9	56	9	56
Source of variation			---------- *P*–values ----------			
Breeder feed restriction		0.357	0.300	0.219	0.234	0.208
Incubation profile		0.922	<0.001	0.030	<0.001	0.029
Feed restriction x Incubation profile		0.713	0.884	0.978	0.846	0.968

[1]Values are means of 12 pens per treatment combination with 7 chickens Cobb 500, for hatch and 7 d, and 24 for 21 d
[2] EDF – Every-day feeding program
[3] SAD – Skip-a-day feeding program
[4] S – Standard incubation temperature
[5] LH – Low-high incubation temperature
[a,b] Means in a column not sharing a common superscript are significantly different ($P<0.05$) by Student's t test

Table III – 3. Effect of feed restriction programs and incubation temperature profiles on chicken progeny chicken progeny feed intake (FI), and feed conversion ratio (FCR) of 60-wk-old Cobb 500 broiler breeders

Breeder feed restriction	Incubation temperature	FI (g)			FCR (g:g)	
		0-7 d	7-21 d	0-21 d	0-7 d	0-21 d
EDF		170	1060	1230	1.20	1.32
SAD		168	1078	1249	1.19	1.33
	Standard	172	1080	1254[a]	1.17[b]	1.31[b]
	Low - high	166	1058	1225[b]	1.22[a]	1.34[a]
EDF	Standard	171	1082	1253	1.17	1.31
	Low - high	169	1038	1207	1.23	1.33
SAD	Standard	173	1078	1255	1.17	1.31
	Low - high	163	1078	1243	1.21	1.35
Pooled SEM		13	46	45	0.04	0.04
Source of variation		---------- *P*–values ----------				
Breeder feed restriction		0.551	0.229	0.182	0.473	0.531
Incubation profile		0.098	0.124	0.046	0.002	0.028
Feed restriction x Incubation profile		0.277	0.117	0.242	0.628	0.381

[1]Values are means of 12 pens per treatment combination with 7 chickens Cobb 500, for hatch and 7 d, and 24 for 21 d
[2] EDF – Every-day feeding program
[3] SAD – Skip-a-day feeding program
[4] S – Standard incubation temperature
[5] LH – Low-high incubation temperature
[a,b] Means in a column not sharing a common superscript are significantly different ($P<0.05$) by Student's t test

Table III - 4. Effects of breeder feeding program and incubation temperature on *stratus corneum*, epidermis and dermis length at hatch, 7 and 22 d

Breeder feeding program	Incubation temperature	*Stratus corneum*			Epidermis			Dermis		
		Hatch	7 d	22 d	Hatch	7 d	22 d	Hatch	7 d	22 d
		---------- µm ----------								
EDF		27	51[b]	150	61	135	201	180[b]	488	1101
SAD		29	61[a]	141	61	150	187	217[a]	486	1113
	S	30	58	146	63	147	187	199	524[a]	1184[a]
	LH	27	55	145	59	138	200	199	451[b]	1029[b]
EDF	S	30	53	158[a]	66	151[ab]	211[a]	189	504	1176
EDF	LH	25	49	143[ab]	56	119[b]	191[ab]	172	472	1026
SAD	S	29	62	133[b]	60	144[ab]	164[b]	208	543	1193
SAD	LH	29	60	148[ab]	62	156[a]	209[a]	226	429	1035
Pooled SEM		5	15	29	12	28	38	50	116	257
Source of variation		---------- *P*–values ----------								
Breeder feeding program		0.250	0.030	0.163	0.991	0.069	0.093	0.023	0.966	0.849
Incubation temperature		0.089	0.552	0.964	0.339	0.231	0.125	0.999	0.040	0.008
BFP x IT		0.117	0.939	0.032	0.130	0.019	0.001	0.268	0.238	0.930

[1]Values are means of 12 chickens Cobb 500, for hatch and 7 d, and 24 for 22 d
[2] EDF – Every-day feeding program
[3] SAD – Skip-a-day feeding program
[4] S – Standard incubation temperature
[5] LH – Low-high incubation temperature
[a,b] Means in a column not sharing a common superscript are significantly different ($P<0.05$) by Student's t or Tukey's test

Table III - 5. Effects of breeder feeding program and incubation temperature on *stratus corneum*, epidermis and dermis area at hatch, 7 and 22 d

Breeder feeding program	Incubation temperature	*Stratus corneum*			Epidermis			Dermis		
		Hatch	7 d	22 d	Hatch	7 d	22 d	Hatch	7 d	22 d
		---------- $\mu m^2 \times 10^3$ ----------								
EDF		12.9[b]	38.6	253.4	25.6	123.4	302.7	51.2[b]	198.9	1107.3
SAD		16.5[a]	46.8	237.4	27.4	135.7	291.0	63.4[a]	203.0	1148.1
	S	13.6[b]	45.7	244.0	27.3	147.4[a]	289.1	55.6	220.0[a]	1178.7
	LH	15.8[a]	39.6	246.8	25.8	111.7[b]	304.7	59.0	181.8[b]	1076.7
EDF	S	13.3[b]	43.5	270.1[a]	27.5	149.2	325.6[a]	47.1	224.6	1204.8
EDF	LH	12.5[b]	33.6	236.8[ab]	23.7	97.5	279.8[ab]	49.5	173.2	1009.8
SAD	S	13.8[b]	48.0	218.0[b]	27.0	126.0	252.6[b]	58.3	215.5	1152.6
SAD	LH	19.2[a]	45.5	256.9[ab]	27.9	145.5	329.5[a]	68.5	190.5	1143.6
Pooled SEM		3.1	15.6	68.3	7..2	52.9	76.7	16.3	60.5	297.8
Source of variation		---------- *P*–values ----------								
Breeder feeding program		0.001	0.070	0.480	0.429	0.422	0.664	0.020	0.816	0.656
Incubation temperature		0.033	0.203	0.827	0.428	0.030	0.254	0.500	0.036	0.118
BFP x IT		0.005	0.454	0.032	0.325	0.299	<0.001	0.181	0.459	0.167

[1]Values are means of 12 chickens Cobb 500, for hatch and 7 d, and 24 for 22 d
[2] EDF – Every-day feeding program
[3] SAD – Skip-a-day feeding program
[4] S – Standard incubation temperature
[5] LH – Low-high incubation temperature
[a,b] Means in a column not sharing a common superscript are significantly different ($P<0.05$) by Student's t or Tukey's test

Table III - 6. Effects of breeder feeding program and incubation temperature on *papillae* height, width and surface area at hatch, 7 and 22 d and *papillae* number at 22 d

Breeder feeding program	Incubation temperature	Height			Width			Surface area		Papillae number
		Hatch	7 d	22 d	Hatch	7 d	22 d	Hatch	22 d	22 d
		---------- µm ----------						---- mm^2 ----		
EDF		270[b]	678	1499	341	526	1270	0.060	1.76	41
SAD		310[a]	714	1459	341	564	1260	0.060	1.59	43
	S	288	736	1563[a]	344	568	1242	0.060	1.65	43
	LH	292	656	1394[b]	338	522	1288	0.062	1.70	42
EDF	S	287[ab]	714	1611	344	574	1272	0.063	1.90[a]	39[b]
EDF	LH	253[b]	641	1386	338	478	1268	0.056	1.62[ab]	44[ab]
SAD	S	288[ab]	757	1515	344	562	1213	0.061	1.39[b]	47[a]
SAD	LH	332[a]	670	1403	339	566	1307	0.059	1.78[a]	39[b]
Pooled SEM		62	151	286	33	97	170	0.013	0.41	7
Source of variation		---------- *P*–values ----------								
Breeder feeding program		0.044	0.438	0.535	0.967	0.185	0.790	0.816	0.057	0.198
Incubation Temperature		0.812	0.083	0.009	0.617	0.117	0.225	0.197	0.544	0.378
BFP x IT		0.049	0.815	0.366	0.919	0.086	0.191	0.378	<0.001	<0.001

[1]Values are means of 12 chickens Cobb 500, for hatch and 7 d, and 24 for 22 d
[2] EDF – Every-day feeding program
[3] SAD – Skip-a-day feeding program
[4] S – Standard incubation temperature
[5] LH – Low-high incubation temperature
[a,b] Means in a column not sharing a common superscript are significantly different ($P<0.05$) by Student's t or Tukey's test

Table III - 7. Pairwise correlations between residual yolk at hatch and BW with the histological measurements at hatch, 7 and 22 d

Variable correlation		BW			Residual yolk at hatch
		Hatch	7 d	22 d	
		---------- *r* ----------			
	Stratus corneum	-0.03	0.09	-0.05	0.05
Lenght	Epidermis	-0.05	0.18	0.18	0.16
	Dermis	0.07	0.13	0.19	0.16
	Stratus corneum	-0.06	0.16	0.02	-0.01
Area	Epidermis	-0.05	0.09	0.20	0.12
	Dermis	0.07	0.12	0.30*	0.16
Height		0.04	0.15	0.22*	0.16
Width		-0.10	0.08	0.15	0.03

* Significant at $\alpha<0.05$ level

CHAPTER IV

Effects of Incubation Temperatures and Trace Mineral Sources on Footpad Skin Development

ABSTRACT

An experiment was conducted to evaluate the effects of two incubation temperatures profiles (**TEM**) and two trace mineral (**TM**) sources on broiler performance and footpad skin development. A total of 1000 Ross 708 eggs were incubated following two TEM profiles, a standard (S) eggshell T (38.0^{o}C) temperature and early-low late-high TEM (LH). This second profile had low (36.9^{o}C) TEM for the first 3 d, and S until the last 3 d where TEM was elevated (38.9^{o}C). At hatch, 15 males and 15 females from each TEM were selected and footpads sampled for histology. Additionally, 168 males per TEM were placed in 24 battery cages with 7 chickens each. The 48 cages were assigned to two TM dietary treatments, one with inorganic (ITM) sources of Zn, Cu, and Mn and other with an organic (OTM) source using lower inclusion levels. Individual BW and group FI were obtained at 7 and 21 d of age to obtain BW gain and FCR. At 7 and 21 d 2 chickens were sampled for footpads. Histological analysis assessed thickness and area of *stratus corneum* (**SC**), epidermis and dermis. Data were analyzed as a CRBD in a 2x2 factorial arrangement of treatments, with TEM and sex as main factors for hatch and TEM and TM diets for 7 and 21 d data. The LH chickens were heavier than S chickens at hatch, but with more residual yolk. However, S TEM chickens were heavier at 7 and 21d. The S TEM reduced FCR. The ITM diets increased BW gain at 7 d. *Papillae* dermis parameters were increased by S TEM at hatch. Additionally, females had thicker and increased area of SC. At 7 d, SC height and area were increased by S TEM. At 21d OTM diets increased dermis height and area. In conclusion TEM affect footpad skin structure and broiler performance. Additionally, reduction of TM levels when using an organic source did not affect chicken live performance and increased dermis development.

Key words: footpad development, broilers, incubation, trace minerals, collagen

INTRODUCTION

The importance of chicken paws in poultry industry had increased in the past yr due to the high demand from the Asian markets for this product (US Poultry & Egg Export Council, 2009). Therefore, to be able to guarantee a good quality of chicken feet at market age have become one of the principal concerns of poultry companies in order to increase revenues (Shepherd and Fairchild, 2010). The value of chicken feet is determined by the size of paws, and its overall health. Paw health is mainly associated with footpad dermatitis (**FPD**) with most of the feet condemnations due to the presence of these lesions (Berg, 1998; Breuer et al., 2006; Shepherd and Fairchild, 2010). Another feature that enhances importance of FPD is its relationship with animal welfare and performance. FPD has been taken as major criteria on broiler welfare audits in Europe and United States (Berg, 2004; Berg and Algers, 2004).

Several parameters have been reported to be involved on the onset of these lesions. Litter condition, nutrition and genetics are well recognized factors that are involved in development of foot lesions (Mayne, 2005; Shepherd and Fairchild, 2010; Wu and Hocking, 2011; De Jong et al., 2012). However, these parameters are common to all individuals in a flock where several degrees of FPD affection are observed. Differences on footpad skin structure development may explain the different susceptibility between individuals. The footpad skin of chicken feet is covered with multiple reticular scales with papillary form (Prin et al., 2004). The structure of these *papillae* is characterized by the presence of a thick *stratus corneum* (**SC**), followed by epidermis and dermis layers (Bacha Jr. and Bacha, 2000). On FPD, thickening of *stratus corneum* and acanthosis are

observed with a concomitant papillary growth and formation of secondary *papillae* (Pass, 1989; Martland, 1984; Whitehead, 1990).

Trace minerals (**TM**) such as zinc, copper and manganese are important components of the diets since they intervene on development of several chicken body structures (McDowell, 2003; Manangi et al., 2012). The TM zinc is found in every tissue of the bone and high concentrations of it are observed in bones, skin and feathers (Leeson and Summers, 2001). Adequate bioavailability of this mineral is important since the processes of collagen synthesis, keratinization and cell replication require zinc as cofactor (Sunde, 1972; McDowell, 2003). Another TM involved with skin health is copper. This mineral is an essential cofactor on collagen and elastin cross-linking (Zhao et al., 2010). Manganese is also important for animal structural development since it is involved in anti-oxidant functions and bone development (McDowell, 2003). The source of these minerals has been reported by several authors to be related with FPD condition in chickens (Burger et al., 1984; Hess et al., 2001; Zhao et al., 2010; Saenmahayak et al., 2010; Manangi et al., 2012). This relationship is thought to be mainly associated with differences in bioavailability. Organic sources usually suffer less antagonistic reactions with other dietary compounds in the gastrointestinal tract being better absorbed and ready to use by the animals (Zhao et al., 2010). Some authors observed an improvement of paw quality when using organic sources of zinc, manganese and cupper (Hess et al., 2001; Zhao et al., 2010; Saenmahayak et al., 2010; Manangi et al., 2012). Manangi et al., (2012) in one experiment observed that when using organic sources and a reduction of 100ppm Zn, 125ppm Cu and 90ppm of Mn of inorganic source to 32ppm Zn, 8ppm Cu and 32ppm of Mn chelated with HMTBA, FPD incidence was reduced.

Incidence of FPD has also been reported to be related with hatchery conditions (De Jong et al., 2012). These authors reported that different hatcheries are associated with different FPD severities in chickens at market age. Furthermore, environmental conditions during the incubation period have been reported to affect embryo development (Leksrisompong et al., 2007; Yalçin et al., 2008 Oviedo-Rondón et al., 2009; Molenaar et al., 2011). High incubation temperatures can affect embryo metabolism and induce changes in body structure of chickens (Leksrisompong et al., 2007; Yalçin et al., 2008). Yalçin et al. (2008) observed that embryo beak development at 18th day of incubation was negatively affected when using high temperatures (38.5°C) between the 10th and 18th day of incubation. Therefore incubation conditions may impact the development of keratinaceous tissues of the chicken embryos.

Considering that FPD is a major concern related with animal welfare and company profitability, the objective of this experiment was to evaluate the effect of incubation conditions and dietary TM sources on the development of footpad skin of chickens. Therefore, the major histomorphological alterations on the three layers of broiler footpads – SC, epidermis and dermis – were evaluated at hatch, 7 and 21 d of age and collagen content of footpad skin quantified at 21 d.

MATERIAL AND METHODS

Incubation Treatments

A thousand eggs coming from 29 wk old Ross 708 breeders were randomly divided in two groups of 500, numbered and weighed. Afterwards, each group was set randomly in four incubator trays (~120 eggs per tray) and placed into two ChickMaster machines (G18) for incubation. Each setter was assigned with an incubation temperature profile

(**TEM**) treatment and worked with a relative humidity of 49% and a 45° from the vertical every h. The TEM consisted of a standard (**S**) and a low-high (**LH**) TEM. The S treatment was obtained by setting the setter temperature that allow normal eggshell temperatures (37.5 - 38°C) during the first 18.5 d of incubation. The LH treatment consisted of a TEM that was set to obtain low eggshell temperatures (36.9°C) during the first 4 d of incubation, normal temperatures (37.5 - 38°C) between the 4^{th} and 17^{th} day of incubation and high temperatures (38.9°C) until hatching. Eggs were candled and transferred at 18.5 d of incubation to two separate hatchers maintaining each incubation treatment. At hatch 12 chicks from each TEM were randomly selected, weighed and sampled to obtain skin footpad using a circular biopsy punch with 5mm of diameter, on both feet. The tissues were fixed in a square of paper to avoid shrinkage and stored in a 10% formalin solution for 48 h. After fixation tissues were transferred to a 70% alcohol solution for further histological processing.

Dietary Treatments

At hatch 168 males per TEM were randomly selected and identified with a neck tag. These chicks were then placed in 48 Petersime cages with 7 broilers on each. Two TM dietary treatments were assigned randomly to the cages having in the end a total of 12 cages per treatment combination (84 chickens/treatment). The dietary treatments consisted in two diets with different TM sources, inorganic (**ITM**) vs. organic (**OTM**), and levels of inclusion. The ingredient inclusion and nutrient matrix of the crumbled diet are described in detail in the table IV-1. The two diets were formulated according to the genetic line recommendations only differing in the zinc, manganese and copper levels. The premix of the ITM diet was formulated to obtain 120 ppm of supplemental zinc as

ZnSO4, 10 ppm of supplemental copper as CuSO4, and 120 ppm of supplemental manganese as MnSO4. The OTM treatment was obtain by replacing the previous mentioned ITM sources for minerals chelated with bis(-2-hydroxy-4-methylthio) butanoic acid (**HMTBA**). The OTM premix was formulated to obtain 32 ppm of supplemental Zn as Zn-$(HMTBA)_2$, 8 ppm of supplemental Cu as Cu-$(HMTBA)_2$, and 32 ppm of supplemental Mn as Mn-$(HMTBA)_2$. The diets were adjusted to maintain the same levels of methionine using HMTBA. Both diets were fed *ad libitum* up to 21 d. Lighting program and room temperature were checked daily and followed the commercial practices. All practices regarding bird care were approved by the Institutional Animal Care and Use Committee of North Carolina State University.

Data Collection

Individual chicken BW and feed weight were recorded at hatch, 7 and 21 d to calculate BW gain, feed intake and FCR for the periods 0 to 7 and 0 to 21 d. Mortality was recorded daily for adjustment of feed conversion ratio. At hatch 15 males and 15 females from each treatment combination were randomly selected, euthanized by cervical dislocation, weighed with and without residual yolk and both footpad skins collected using a circular biopsy punch with 5 mm of diameter. Footpads were fixed in a dry paper to avoid shrinkage and stored in a 10% formalin solution for 48 h. Afterwards tissues were washed and transferred for a 70% alcohol solution. At 7 and 21 d, one and two birds respectively, were randomly selected per cage, weighed, and sacrificed by cervical dislocation. Footpad skin of the 7 and 21 d birds were sampled according to the same procedure described for hatch. At 21 d the footpads were collected using a scalpel for *papillae* surface area evaluation. For the three sampling times, the right foot footpad

tissues were processed for histology, cut in cross sections of 3 – 5 mm of thickness, and stained using the Masson's trichrome dye. The slides were then analyzed using an optical microscope and photographs of *papillae* present in the footpads were taken using a camera attached to it. The magnification of 200x, 100x, and 40x for hatch, 7 d and 21 d was used respectively.

Each photo was histologically analyzed using Image Tool Software (Version 3.0, University of Texas Health Science Center, San Antonio, TX, USA). Thickness and total area of SC, epidermis and dermis and total height and width of *papillae* were accessed (Figure IV - 1). In each footpad five *papillae* were evaluated and measured in three different points with in the *papillae* for each measurement. The left footpad from hatch, 7 and 21 d were used to access papillae surface area at both ages, using a stereoscopic microscope. Zoom of 2.3x was used for hatch, 2.1x for 7 d and 1.8x for 22 d. Additionally, at 21 d, three broilers per cage were sacrificed and all the footpads were collected, pooled and frozen at -20°C to perform collagen analysis. The collagen content was determined by hydroxyproline quantification. This amino acid has the peculiar feature of be mainly present in collagen (Ignat'eva et al., 2007; Salim et al., 2010). Considering this, hydroxyproline has been used to determined collagen content of various biological tissues (Ignat'eva et al., 2007). The hydroxyproline content was determined following a procedure similar to what described by Ignat'eva et al. (2007) and Salim et al. (2010). The analyses consisted in a hydrolyzation of 10mg of the footpad pooled tissues with 100µL of 12N hydrochloric acid at 120°C in a hotplate for 3 h. The samples were then mixed with chloramine T plus oxidation buffer, incubated at room temperature for 5 minutes and after, solutions of 4-Dimethylaminobenzaldehyde and Perchloric Acid/Isopropanol, were added. The plate was incubated at 60°C for 90 minutes and

absorbance measured at 560nm using a spectrophotometer. The obtained absorbance values were plotted against the concentration of a standard hydroxyproline, and the presence of hydroxyproline in footpad tissues was determined from the standard curve. The total collagen content of the footpad skin was determined by multiplication of the hydroxyproline results by 7.5 (Bonifer and Froning, 1996).

Data Analysis

Footpad hatch data were analyzed as a randomized complete block design in a 2x2 factorial arrangement with TEM and sex as main factors. The performance and 7 and 21 d footpad measurements data were analyzed as the hatch data however, the main factors used were TEM and dietary TM source. In order to obtain normality some data had to be transformed natural logarithms. At hatch, *papillae* height and dermis length and area were transformed. Epidermis length, dermis area and *papillae* height and width of 21 d measurements were also transformed. All data were analyzed with JMP 10 (SAS Inst. Inc., Cary, NC) software.

RESULTS

Performance

The performance data of the chickens placed in the cages (Table IV – 2) indicated that at hatch chickens incubated with LH TEM were heavier than chickens incubated with S TEM ($P<0.01$). Additionally this same tendency ($P<0.07$) was observed in the chickens sampled. However, when evaluating residual yolk we observed ($P<0.01$) that chickens incubated with LH TEM had higher (8.95%) percentage of residual yolk in comparison with chickens incubated with S (7.40%) TEM (Table IV – 2). No differences ($P>0.05$)

between sexes were observed on BW and yolk utilization. On the other hand at 7 and 21 d the chickens incubated with S temperatures were heavier (P<0.05) than the ones incubated with LH. In addition a reduction of FCR from the 0 to 7 and 0 to 21 d was obtained when the chickens were incubated with S temperatures (Table IV – 4). The dietary treatments had an effect (P<0.05) on BW and BW gain at 7 d but these differences were no longer observed at 21 d. When chickens were fed ITM diets both BW and BW gain were higher in comparison with OTM diets. Additionally, FI was higher (P<0.05) on ITM diets at 7 d. No differences (P>0.05) were observed on FCR between diets.

Footpad Development at Hatch

Based on the histological measurements (Table IV – 5) at hatch, there was an effect (P<0.05) of TEM observed on epidermis area, dermis thickness and area. When chickens were incubated with S TEM, an increased epidermal area was observed. In addition, chickens incubated with LH TEM had a reduced dermis layer development. The SC was also affected (P<0.05) by bird sex. Females had thicker and larger SC layer when compared with males. The overall structure of *papillae*, height and width, were affected (P<0.05) by TEM. Exposure to improper incubation temperatures reduced the height and width of the *papillae*. An interaction (P<0.01) of treatments was observed on *papillae* surface area. A difference between sexes was obtained when eggs were incubated using S TEM with the males having lower *papillae* surface area. No significant pairwise correlations (Table IV – 8) of residual yolk and BW and footpad skin layers parameters were observed.

Footpad Development at 7 d

At 7 d a TEM effect ($P<0.05$) was observed on SC thickness and area (Table IV – 6 and Table IV – 7). The S TEM increased these parameters by the end of the first wk of life. No other effects ($P>0.05$) of treatments or pairwise correlations with BW were obtained on footpad structures at 7 d.

Footpad Development at 21 d

The 21 d measurements demonstrated an effect ($P<0.05$) of TM source on the dermis thickness and area. Birds fed diets with OTM had thicker and increased dermis area. Furthermore, OTM diets also increased *papillae* height in comparison with birds fed ITM diets. On pairwise correlations between footpad skin layers and BW, weak ($r<0.26$) significant ($P<0.05$) correlations were observed on SC thickness and area and epidermis area. No differences ($P>0.05$) between treatment combinations were obtained on footpad skin collagen content.

DISCUSSION

In past studies several authors reported the effects of temperature during incubation on embryo development and chicken performance post-hatch (Christensen et al., 2005; Leksrisompong et al., 2007; Lourens, et al. 2007; Hulet et al., 2007; Molenaar et al., 2011). Molenaar et al. (2011) reported a decrease on yolk-free BW at hatch when eggs were exposed to high eggshell temperatures (>38.9ºC) after the day 7 of incubation. Furthermore, the authors argue that this deleterious effect on BW was still observed at 21 and 42 d of age. In the present study, it was observed a negative effect of the LH TEM at 21 d though, when evaluating yolk-free BW at hatch no differences between the

treatments were observed. Nevertheless, our results are in agreement with what was described by Hulet et al. (2007). These authors tested the effect of exposure to different eggshell temperatures (37.5, 38.6, and 39.7 ºC) in the hatcher on chicken performance. Similarly to the data presented here in, Hulet et al. (2007) observed that chickens incubated with high eggshell temperatures (39.7 ºC) during the four last d of incubation were heavier than the chickens incubated with middle (38.6ºC) and low (37.5 ºC) temperatures at hatch. In the experiment presented here in, chickens incubated with the LH TEM were 1g heavier than the ones incubated with S TEM. However, when looking to the yolk-free BW data we observed that this difference is no longer observed. Furthermore, on the residual yolk evaluation, LH TEM increased residual yolk by 1.55%. Therefore, the differences on BW observed at hatch in the present experiment are related to reduced yolk utilization by chickens embryos incubated with LH TEM. Hulet et al. (2007) also reported that chickens incubated with middle temperatures (38.6 ºC) were heavier at 21, 35 and 40 d of age. Additionally, the chickens hatched with high incubation temperatures were reported to have higher FCR. The LH TEM used in the present experiment had similar effect on chicken performance reducing BW gain and increasing FCR at 7 and 21 d.

In an experiment conducted by Manangi et al. (2012), the authors tested the same TM reduction strategy (32ppm Zn, 8ppm Cu, and 32ppm Mn), using the same OTM sources as the one used in this experiment. These authors did not observe any difference on BW at 51 d when comparing with birds fed diets with ITM sources and normally used inclusion levels (100ppm Zn, 125ppm Cu, and 90ppm Mn). In the present experiment no differences were found at 21 d on BW and performance. However, diets with OTM sources resulted in decreased BW at the end of the first wk. Therefore chickens might be

more susceptible to the reduction or TM source during the first wk of life, though they can recover by the end of the 3rd wk.

The effects of incubation temperature on the skin layer parameters at hatch indicate that the treatments did have an effect on skin development during the embryo development. Footpad lesions are characterized by hyperplasia of epidermis and also by thickening of SC (Pass, 1989). Even though, no effects were observed on epidermis length the trend observed on epidermis area indicates that incubation temperatures might have an influence on FPD onset early in life. Additionally, the higher proliferation of SC obtained on female chicks might explain what some authors described as a female predisposition for higher incidence of FPD (Kjaer et al., 2006). The incubation temperatures effect at hatch is also a strong indicator of how important incubation temperature is for skin development. The strength of skin has been reported to be correlated with dermis thickness (Christensen et al., 1994). Furthermore, the dermal layer is where skin collagen is found (Ramshaw et al., 1986; Christensen et al., 1994). Therefore, reduction in thickness and area of dermis may be correlated with low collagen content and consequently result in weaker footpad skin. On *papillae* height the increased *papillae* observed on chickens incubated with S TEM is probably related with the thicker dermis layer obtained when using this incubation temperature profile. In this experiment the *papillae* height was measured from the bottom of dermis layer to the top of SC, and consequently, due to the increased dimensions of the dermal layer in relation to SC and epidermis, it has a higher influence on *papillae* height. The reduction of width induced by LH TEM also revealed that *papillae* of birds incubated with stress full conditions have a thinner base. Furthermore, the interaction between incubation profile and sex demonstrate that structurally the top of *papillae* on females is larger than the males when incubated

with S TEM. At 7 d, the thicker SC observed on S chickens may be related with the higher epidermis values observed at hatch. Nevertheless, the treatment effects on SC and epidermis were no longer observed at 21 d.

The only effect of the TM diets was observed on dermis thickness and area at 21 d. Usage of TM from an organic source with reduction of the inclusion levels increased dermis structure overall. As mentioned previously this might be an indicator of stronger skin. However, when quantifying footpad skin collagen, no differences were observed between the diets. The authors were not able to obtain reports in the literature pertaining to collagen quantification of footpads. Nevertheless, Salim et al. (2010) reported an increase on collagen content and thickening of dermis on broiler back skin when feeding diets with an organic zinc source (Bioplex Zn, Alltech Inc., Nicholasville, KY, USA) in comparison with an inorganic source ($ZnSO_4$), at 40 ppm of inclusion. The improvement on dermis development observed may explain the reduction on severe paw lesions described by Manangi et al. (2012) when feeding diets similar to the ones used in the experiment reported here in. These authors compared FPD scores of broilers fed diets with 100 ppm zinc, 125 ppm copper and 90 ppm of manganese of an inorganic source against broilers fed 32 ppm zinc, 8 ppm copper and 32 ppm of manganese chelated with HMTBA. They reported an increase of paws without or with minor lesions and a decrease of paws with severe lesions on the birds fed diets with trace minerals chelated with HMTBA. Considering the hatchery effect on FPD severity, reported by De Jong et al. (2012), together with the footpad skin structural changes induced by incubation temperatures in this experiment, it is plausible that incubation conditions affect broiler susceptibility to develop FPD.

In conclusion, the S TEM increased BW gain and feed efficiency at three wk of age. On the other hand LH TEM decreased *papillae* width and dermis development at hatch which might related with weaker footpad skin and *papillae* increasing consequently the susceptibility to develop FPD. Furthermore, reduction of zinc, copper and manganese when using an OTM source did not affect performance and increased footpad skin dermis development which might be related with reduction of FPD severity in broiler.

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Table IV-1. Composition (%) and formulated nutrient contents of the basal starter broiler diet and the mineral inclusion of the inorganic and organic diets

Ingredients	Starter basal 1-21 d
	%
Corn	47.25
Soybean meal, 48%	35.53
Distillers dried grains with solubles	6.63
Poultry fat	5.08
Salt (NaCl)	0.44
Limestone	1.42
Dicalcium phosphate, 18.5%	1.12
Alimet[1]	0.23
L-lysine-HCl, 78,8%	0.15
Choline chloride, 60%	0.20
Sodium bicarbonate	0.15
L-threonine, 98%	0.03
Coccidiostat[2]	0.05
Mineral premix[3]	0.20
Vitamin premix[4]	0.10
Phytase[5]	0.02
Filler[6]	1.4
Total	100.00
Nutrient composition	
ME, kcal/kg	3,050
CP, %	22.85
Calcium, %	0.90
Total phosphorus, %	0.64
Available phosphorus, %	0.38
Digestible. lysine, %	1.20
Digestible total sulfur amino acids, %	0.84
Digestible threonine, %	0.78
Digestible tryptophan, %	0.24
Sodium, %	0.25
Potassium, %	0.98
Chloride, %	0.32
Dietary electrolyte balance, mEq/100 g	274

[1]Alimet (Novus International Inc., St. Charles, MO), feed supplement providing 88% Met activity.

[2]Coban 90® 45 (Monesin Sodium), Elanco Animal Health, Greenfield, IN.

[3]The trace mineral premix supplied the following per kilogram of diet: 80 ppm of Fe as Iron Sulfate, 2.5 ppm of I as Calcium Iodate, 1 ppm Co as Cobalt Sulfate. The inorganic trace mineral mix had 120 ppm of Zn as Zinc Sulfate, 10 ppm of Cu as Copper Sulfate, 120 ppm of Mn as Manganese Sulfate. The organic trace mineral mix had 32 ppm of Zn as Zn-$(HMTBA)^2$, 8 ppm of Cu as Cu-$(HMTBA)^2$, and 32 ppm of Mn as Mn-$(HMTBA)^2$. The total methionine activity [2-hydroxy-4-(methylthio) butanoic acid; HMTBA] contribution of 0.036% from Zn-$(HMTBA)^2$ (16% Zn, 80% HMTBA), Cu-$(HMTBA)^2$ (15% Cu, 78% HMTBA), and Mn-$(HMTBA)^2$ (13% Mn, 76% HMTBA) was balanced in the inorganic diet to maintain an equimolar quantity of HMTBA across both treatments.

[4]Vitamins from premix provided per kilogram of premix: vitamin A, 18,739,292 IU; vitamin D_3, 6,613,868 IU; vitamin E, 66,139 IU; vitamin B_{12}, 33 mg; riboflavin, 22,046 mg; niacin, 88,185 mg; d-pantothenic acid, 30,865mg; menadione, 3,968 mg; folic acid, 2,646 mg; vitamin B_6, 7,716 mg; thiamine, 5,512 mg; biotin, 176 mg.

[5]Ronozyme® P CT at 185 g/ton to provide 930 FYT (DSM Nutritional Products, Parsippany, NJ).

[6]Celite, Celite Corp., Lompoc, CA.

Table IV-2. Effect of incubation temperature profiles and trace mineral sources on BW and BW gain (BWG) of male Ross 708 broilers

Incubation temperature	Trace mineral source	BW (g)			BWG (g)	
		0 d	7 d	21 d	0-7 d	0-21 d
Standard		40.7[b]	149[a]	932[a]	108[a]	891[a]
Low - high		41.7[a]	146[b]	908[b]	104[b]	789[b]
	Inorganic	-	150[a]	929	109[a]	888
	Organic	-	144[b]	911	103[b]	869
Standard	Inorganic	-	152	940	112	899
	Organic	-	146	924	105	883
Low - high	Inorganic	-	148	919	106	877
	Organic	-	143	897	102	855
		1.1	6	36	6	36
Source of variation			---------- *P*–values ----------			
Incubation temperature		0.003	0.048	0.031	0.010	0.026
Trace mineral source		-	0.001	0.094	0.003	0.093
Incubation temperature x TM source		-	0.646	0.776	0.583	0.766

[1]Values are means ± SEM of 12 pens per treatment combination with 7 chickens for hatch and 7 d and, 6 for 21 d
[a,b] Means in a column not sharing a common superscript are significantly different ($P<0.05$) by Student's t test.

Table IV-3. Effect of incubation temperature profiles and sex on BW with and without yolk and residual yolk of Ross 708 broilers at hatch

Incubation temperature	Sex	BW (g)		Residual yolk (%)
		With yolk	Without yolk	
Standard		37.0	34.2	7.40 [b]
Low – high		38.0	34.5	8.95 [a]
	Male	37.1	34.1	8.01
	Female	37.7	34.6	8.33
Standard	Male	36.4	33.7	7.32
	Female	37.4	34.6	7.48
Low – high	Male	37.8	34.5	8.71
	Female	38.1	34.6	9.19
Pooled SEM		2.1	1.8	1.9
Source of variation		---------- *P*–values ----------		
Incubation temperature		0.072	0.437	0.005
Sex		0.292	0.378	0.583
Incubation temperature x sex		0.533	0.413	0.720

[1]Values are means ± SEM of 30 broiler chickens per treatment

[a,b] Means in a column not sharing a common superscript are significantly different ($P<0.05$) by Student's t test.

Table IV-4. Effect of incubation temperature profiles and trace mineral sources on FI, and FCR of male Ross 708 broilers

Incubation temperature	Trace mineral source	FI (g)			FCR (g:g)	
		0-7 d	7-21 d	0-21 d	0-7 d	0-21 d
Standard		105	1027	1129	0.97[b]	1.26[b]
Low - high		105	1014	1118	1.00[a]	1.30[a]
	Inorganic	108[a]	1028	1135	0.99	1.29
	Organic	102[b]	1012	1112	0.98	1.27
Standard	Inorganic	109	1041	1150	0.98	1.28
	Organic	101	1012	1108	0.96	1.24
Low - high	Inorganic	106	1015	1120	1.01	1.30
	Organic	104	1012	1115	1.00	1.30
Pooled SEM		8	46	50	0.05	0.04
Source of variation			---------- *P*–values ----------			
Incubation temperature		0.926	0.326	0.444	0.027	0.001
Trace mineral source		0.026	0.239	0.118	0.401	0.157
Incubation temperature x TM source		0.215	0.338	0.210	0.855	0.139

[1]Values are means ± SEM of 12 pens per treatment combination with 7 chickens for hatch and 7 d and, 6 for 21 d
[a,b] Means in a column not sharing a common superscript are significantly different ($P<0.05$) by Student's t test

Table IV-5. Effect of incubation temperature profiles and sex on *stratus corneum*, epidermis and dermis development and *papillae* structure at hatch

Incubation temperature	Sex	*Stratus corneum*		Epidermis		Dermis		*Papillae*		Surface area
		Length	Area	Length	Area	Length	Area	Height	Width	
		(µm)	(µm^2×10^3)	(µm)	(µm^2×10^3)	(µm)	(µm^2×10^3)	-- (µm) --		(mm^2)
Standard		36	16.0	62	22.4	144^a	30.1^a	243^a	272^a	0.092
Low - high		36	15.0	64	19.4	116^b	21.9^b	209^b	236^b	0.093
	Male	34^b	14.4^b	63	20.4	118	22.5	207	248	0.087^b
	Female	38^a	16.5^a	63	21.5	142	29.5	244	260	0.098^a
Standard	Male	34	14.7	62	21.7	141	28.7	237	267	0.081^b
Standard	Female	37	17.2	63	23.1	148	31.6	248	276	0.103^a
Low - high	Male	33	14.0	64	23.1	94	16.4	177	228	0.093^{ab}
Low - high	Female	38	15.9	64	19.8	138	27.3	237	244	0.094^{ab}
Pooled SEM		7	3.5	12	5.3	68	15.8	63	46	0.015
Source of variation		---------- *P*–values ----------								
Incubation Temperature		0.906	0.317	0.544	0.053	0.028	0.018	0.030	0.008	0.767
Sex		0.046	0.035	0.951	0.469	0.225	0.096	0.063	0.348	0.005
IT x S		0.569	0.732	0.849	0.866	0.221	0.142	0.105	0.779	0.008

[1]Values are means of 15 chickens Ross 708
[2] IT – Incubation temperature
[3] S – Sex
[a,b] Means in a column not sharing a common superscript are significantly different (P<0.05) by Student's t or Tukey's test

Table IV-6. Effect of incubation temperature profiles and trace mineral sources on *stratus corneum*, epidermis and dermis length at 7 and 21 d

Incubation temperature	Trace mineral source	*Stratus corneum*		Epidermis		Dermis	
		7 d	21 d	7 d	21 d	7 d	21 d
		---------- µm ----------					
Standard		64[a]	121	138	155	566	829
Low - high		55[b]	120	135	155	573	838
	Inorganic	61	121	138	152	587	794[a]
	Organic	58	120	135	158	551	873[a]
Standard	Inorganic	67	124	141	154	572	775
Standard	Organic	60	117	134	156	560	884
Low - high	Inorganic	54	117	136	149	603	814
Low – high	Organic	56	123	135	161	542	862
Pooled SEM		11	19	21	30	80	150
Source of variation		---------- *P*–values ----------					
Incubation temperature		0.005	0.839	0.727	0.957	0.763	0.774
Trace mineral source		0.384	0.917	0.538	0.273	0.125	0.013
IT x TMS		0.178	0.104	0.595	0.416	0.297	0.330

[1]Values are means of 12 chickens Ross 708 for 7 d, and 24 for 21 d
[2] IT – Incubation temperature
[3] TMS – Trace mineral source
[a,b] Means in a column not sharing a common superscript are significantly different ($P<0.05$) by Student's t test

Table IV-7. Effect of incubation temperature profiles and trace mineral sources on *stratus corneum*, epidermis and dermis area at 7 and 21 d

Incubation temperature	Trace mineral source	*Stratus corneum*		Epidermis		Dermis	
		7 d	21 d	7 d	21 d	7 d	21 d
		---------- µm^2 ×10^3 ----------					
Standard		46.8[a]	184.7	95.7	228.8	141.7	664.1
Low - high		40.6[b]	175.1	96.3	223.4	140.3	698.5
	Inorganic	44.2	183.3	97.4	224.9	146.1	635.1[b]
	Organic	43.2	176.5	94.5	227.4	136.0	727.5[a]
Standard	Inorganic	49.3	194.1	97.8	231.6	143.7	605.6
Standard	Organic	44.4	175.4	93.6	226.0	139.7	722.6
Low – high	Inorganic	39.1	172.6	97.1	218.1	148.3	664.6
Low - high	Organic	42.1	177.5	95.4	228.7	132.3	732.3
Pooled SEM		8.9	36.0	19.9	42.6	28.7	185.7
Source of variation		---------- *P*–values ----------					
Incubation temperature		0.021	0.195	0.916	0.540	0.874	0.367
Trace mineral source		0.714	0.355	0.616	0.777	0.241	0.017
IT x TMS		0.143	0.114	0.829	0.360	0.478	0.517

[1]Values are means of 12 chickens Ross 708 for 7 d, and 24 for 21 d
[2] IT – Incubation temperature
[3] TMS – Trace mineral source
[a,b] Means in a column not sharing a common superscript are significantly different ($P<0.05$) by Student's t test

Table IV-8. Effect of incubation temperature profiles and trace mineral sources on *papillae* height, width and surface area at 7 and 21 d and footpad skin collagen content at 21 d

Incubation temperature	Trace mineral source	Height		Width		Surface Area		Collagen[2]
		7 d	21 d	7 d	21 d	7 d	21 d	21 d
		---------- µm ----------						mg/g
Standard		566	1104	501	1057	0.24	1.42	8.45
Low - high		573	1105	490	1043	0.22	1.35	8.93
	Inorganic	587	1063[b]	495	1068	0.23	1.42	8.95
	Organic	551	1147[b]	496	1033	0.23	1.35	8.43
Standard	Inorganic	572	1053	505	1070	0.23	1.48	8.39
Standard	Organic	560	1157	497	1045	0.25	1.35	8.51
Low - high	Inorganic	603	1073	486	1065	0.23	1.35	9.52
Low - high	Organic	572	1138	495	1021	0.21	1.35	8.35
Pooled SEM		80	169	495	126	0.04	0.22	4.00
Source of variation		---------- *P*–values ----------						
Incubation temperature		0.763	0.879	0.538	0.230	0.182	0.160	0.678
Trace mineral source		0.125	0.017	0.982	0.628	0.676	0.185	0.652
IT x TMS		0.237	0.592	0.645	0.637	0.286	0.167	0.581

[1]Values are means of 12 chickens Ross 708 for 7 d, and 24 for 21 d measurements
[2] Values are means of 12 pens per treatment combination with a pool sample of 3 Ross 708 footpads
[3] IT – Incubation temperature
[4] TMS – Trace mineral source
[a,b] Means in a column not sharing a common superscript are significantly different ($P<0.05$) by Student's t test

Table IV - 9. Pairwise correlations between residual yolk at hatch and BW with the histological measurements at hatch, 7 and 21 d

Variable correlation		BW			Residual yolk at hatch
		Hatch	7 d	21 d	
		---------- *r* ----------			
	Stratus corneum	0.25	-0.04	0.24*	0.12
Lenght	Epidermis	0.24	0.11	0.11	-0.02
	Dermis	0.08	0.15	-0.09	0.09
	Stratus corneum	0.25	-0.12	0.26*	0.14
Area	Epidermis	0.24	0.13	0.20*	-0.01
	Dermis	0.01	0.15	-0.02	0.11
Height		0.14	0.14	-0.04	0.11
Width		0.04	0.22	0.17	-0.01

* Significant at $\alpha<0.05$ level

CHAPTER V

Effect of incubation temperature profile on broiler footpad dermatitis

ABSTRACT

A hatchery effect on footpad dermatitis (**FPD**) has been reported in broilers. An study was conducted to evaluate the effects of two incubation temperature profiles (**TEM**) on FPD in broilers at 7, 13 and 28 d. 1000 eggs of Cobb 500 fast-feathering were incubated following two TEM profiles to either maintain eggshell temperature at 38.0^{o}C (S) or have eggshell temperatures similar to the ones observed in multistage machines. This TEM had low (36.9^{o}C) eggshell temperatures for the first 3 d, and standard TEM until the last 3 d when they endured elevated (38.9^{o}C) eggshell temperature. At hatch, 180 broilers per treatment were placed in 15 pens with new pine wood shavings, each with 6 males and 6 females. Additionally, 10 chickens from each TEM and sex had footpads sampled for histology. At 7d incidence of FPD was evaluated. Severity of FPD lesions were assessed at 13 and 28 d using a 4 level scale. Additionally, a pool of litter material was sampled to calculate moisture content. Data were analyzed using a Poisson probability model in a CRBD with TEM and sex as main factors. At 13 and 28 d litter moisture was used as covariate. Furthermore, a linear regression analysis was performed for each TEM to evaluate the variation of FPD with litter moisture. On the histological analysis females had thicker dermis and bigger footpad *papillae* than males. No effects (P>0.05) of treatments were observed at 7d on FPD. At 13d a TEM effect was observed on FPD severity. Chickens incubated with LH INC had higher FPD severity scores. No effect of TEM was observed on FPD at 28 d. However, when litter moisture was not considered, LH chickens had a tendency to have higher FPD scores. Furthermore, LH chickens were more susceptible to litter moisture than S chickens. It was demonstrated that deviations from S TEM can aggravate FPD on the early stages of life and make the birds more prone to litter moisture effects.

Key words: footpad development, broilers, incubation, footpad dermatitis severity

INTRODUCTION

The value of chicken paws in poultry industry have increased in the past 20 years mainly due to the high demand of Asian markets for this product (US Poultry & Egg Export Council, 2009; Philips, 2011). Consequently, a minimum incidence of footpad dermatitis (**FPD**) and improvement of paw integrity have become one of the goals of poultry companies (Shepherd and Fairchild, 2010). Besides the economic importance, paw health has also been regarded as welfare parameter on company audits in Europe and United States (Berg, 2004; Berg and Algers, 2004). Chicken paws are covered with scutate scales on the dorsal part and reticular scales on the plantar surface of the feet that develop during embryogenesis (Sawyer and Knapp, 2003; Prin et al., 2004). The chicken metatarsus is fully covered with a flat two-layered epidermis by the end of the d 9 of incubation (Sawyer, 1972; Bellairs and Osmond, 2005). The development of scales starts by d 12 of incubation and gets completed by d 16 (Sawyer, 1972; Gonzales and Cesario, 2003; Bellairs and Osmond, 2005). Therefore, factors that affect the chicken embryo during incubation may impact skin and scale structure.

Even though FPD have been reported to be mainly impacted by litter conditions (Shepherd and Fairchild, 2010; De Jong et al., 2012) parameters as nutrition, genetic background, sex, season, and incubation have been also related with this condition (Mayne, 2005; Mayne et al., 2007; Shepherd and Fairchild, 2010; Wu and Hocking, 2011; De Jong et al., 2012). Incubation effects on FPD were observed by De Jong et al. (2012) where the authors described different incidence of FPD between hatcheries in

Netherlands. It is well recognized that environmental conditions that embryos are exposed to during incubation affect embryo organogenesis, physiological development and hatchability (Decuypere and Michels, 1992; Yalçin and Siegel, 2003; Tazawa et al., 2004; Oviedo-Rondón et al., 2009; Molenaar et al., 2011). On incubation environment, the incorrect management of incubation temperatures have been reported to affect BW, yolk retention, hormonal regulations and the development of liver, heart and beak (Moraes et al., 2004; Yahav et al., 2004; Leksrisompong et al., 2007; Yalçin et al., 2008; Piestun et al., 2009). The effects of elevated incubation temperatures on embryo beak development observed by Yalçin et al. (2008) at d 18 of incubation indicate that keratinaceous tissues can be impacted by mismanagement of incubation temperatures. Furthermore, high incubation temperatures can alter thyroid plasmatic concentrations (Christensen et al., 2005; Yalçin et al., 2008; Piestun et al., 2009), and these changes have been reported to negatively affect skin thickness in other species under disease status (Sheppard et al., 1966; Safer et al., 2003).

Due to the rise of importance of paw quality for poultry companies and the hypothesis of incubation conditions of having a role on individual animal susceptibility to develop FPD, the goal of this experiment was to evaluate the effect of incubation on FPD severity at 13 and 28 d and footpad skin structure at hatch. Concomitantly, it was also evaluated the effect of litter moisture and bird sex on the aggravation of footpad lesions.

MATERIAL AND METHODS

Incubation Treatments

A total of 1,000 Cobb 500 fast feathering eggs were incubated at the Prestage Department of Poultry Science at North Carolina State University. The eggs were

randomly placed into 24 incubation egg trays that were equally divided into two groups to be incubated according to two incubation temperature profiles (**TEM**). The first TEM was considered the standard (**S**) TEM and was set up to obtain eggshell temperatures of 38.0ºC during the all incubation period. The second TEM was considered the low-early high-late (**LH**) TEM treatment and intended to simulate the variation on eggshell temperatures sometimes observed in incubators with suboptimal conditions, where the eggs are under heated during the early stages and overheated on the last d of incubation. Therefore, the setter was controlled daily to obtain eggshell temperatures of 36.9ºC during the first 3 d of incubation, S eggshell temperatures from 4 to 18 d, and 38.9 ºC during the last 3 d of incubation. The two ChickMaster incubators were programed to operate with a relative humidity of 49% and a 45º egg turning every h. At 18.5 d eggs were candled to remove the infertile ones and transferred to two separate hatchers keeping the TEM.

At hatch, chicks were vent sexed and 10 chicks from each treatment and sex were randomly selected. The BW with and without yolk was record and footpad skin was sampled using a circular 5 mm diameter biopsy punch. The tissues were fixed in a dry paper to avoid tissue shrinkage and deformation and stored in a 10% buffered formalin solution for 48 h. After the 48 h of fixation the samples were transferred to a 70% ethanol solution for further histological processing. The remaining chickens were then transported to the North Carolina State University Research Unit (Raleigh, NC) where the rearing period took place.

Birds and Husbandry

The experiment was performed between February 12th and March 26th of 2013. All the practices regarding animal management were approved by the Institutional Animal Care and Use Committee of North Carolina State University. The project was conducted in a solid wall house with 5 rooms, with exhaust fans and forced air heating system. Each room had 6 pens of 0.86 x 0.89 m of side having new pine-wood shavings as litter material. Each pen was equipped with 1 feeder and nipple drinkers. During the first 5 days of brooding one egg tray with feed was placed in each pen to ease the access to food by the chickens.

At arrival to the farm the chickens from each TEM were randomly divided in groups of six chicks per sex, individually identified with a necktag and group BW was recorded. Afterwards, each pen received a group of each TEM and sex resulting in a total of 12 chickens per pen. The diets fed in the present experiment were corn-soybean based and (Table V - 1) two dietary phases were used: starter - 0 to 14 days, and grower - 15 to 28 days. The starter diets were fed as crumble and grower as pellet. Water and feed were consumed *ad libitum*, though the amount of feed assigned per bird was fixed for the starter phase. The grower diets were consumed *ad libitum* until the end of the experiment. House temperature and lighting program were checked twice a day and followed the commercial practices for this genetic line. All practices regarding bird care were approved by the Institutional Animal Care and Use Committee of North Carolina State University.

Data collection

Male and female group BW per pen were evaluated at 14 and 28 d of age. Additionally, feed consumption was recorded to calculate feed intake and FCR. The footpads collected at hatch were processed for histology, cut in cross sections of 3 – 5 mm of thickness and dyed with Masson's trichrome. Posteriorly, photographs of 5 *papillae* present in the footpads were obtained using an optical microscope with a camera attached using the 200x magnification. The individual *papillae* photos were then analyzed using Image Tool Software (Version 3.0, University of Texas Health Science Center, San Antonio, TX, USA) and thickness and total area of *stratus corneum* (SC), epidermis and dermis were obtained.

The first FPD evaluation was performed at 7 d where each chick was classified as with or without lesion. At 13 and 28 d all birds were evaluated for FPD severity using a four level score system. A footpad with no lesion or morphological alteration was considered level 0. The following levels were graded according the severity being the level 1, 2 and 3 characterized by *papillae* keratosis, brown discoloration and black discoloration with deep lesion respectively. On the two FPD severity evaluations a pool of litter material from 6 points within the pen was sampled to calculate moisture content.

Data Analysis

Performance data were analyzed in a complete randomized block design with the two TEM as treatments. The blocks represented the 5 rooms within the house and were considered as random. For this analysis JMP 10 (SAS Inst. Inc., Cary, NC) was used. Data obtained from the chicken sampled at hatch and the histological measurements were analyzed in a complete randomized design using a 2x2 factorial arrangement with TEM

and sex as main effects. To ascertain any correlation of skin measurements with bird size, pairwise correlations were done for each histological measurement with chicken residual yolk at hatch and BW. The ordinal FPD data was analyzed using a Poisson probability model in a complete randomized block design to model the probabilities of lesions as a function of the factorial effects of TEM and sex. For this analysis the 5 blocks and the pens nested within the treatment combinations were considered a random effect. Due to the correlation of litter moisture with FPD described by some authors (Shepherd and Fairchild, 2010; De Jong et al., 2012), two separate statistical analyses were performed using or not litter moisture as covariate for the 28 d data. Furthermore, two linear regression analyses with a test for significant differences in the slopes were performed for the two TEM treatments to evaluate the variation of FPD score with the litter moisture. The FPD scoring was performed always by the same two evaluators and this factor was added to the model to account for any discrepancy. All the FPD data were analyzed using the GLIMMIX Procedure of SAS (SAS Inst. Inc., Cary, NC). For the BW data pens were the experimental unit and for footpad histological analysis and dermatitis scores each chicken was considered the experimental unit.

RESULTS

Performance

Based on the chicken sampled data, there was an effect ($P<0.01$) of TEM on BW with and without yolk (Table V – 2). Chickens incubated with S TEM were heavier both with and without yolk. No effects ($P>0.05$) of TEM were observed on residual yolk. Nevertheless, sex had an effect ($P\leq0.05$) on yolk utilization by the embryo. Male embryos had less (8.73%) residual yolk in comparison with females (9.91%). Alternatively, based

on the hatch BW of chickens placed at the farm, both male and female chickens from S TEM were lighter ($P<0.01$) than the chickens incubated with LH TEM (Table V – 5). At 14 and 28 d no differences ($P>0.05$) in BW between the TEM treatments were observed on both sexes. However, when evaluating BW gain, females incubated under S TEM gained ($P<0.05$) more weight at 28 d of age in comparison with females incubated with LH TEM (Table V – 6). Additionally, a reduction ($P\leq0.05$) of FCR at 28 d was obtained when chickens were incubated with S TEM (Table V – 7). No effect ($P>0.05$) of treatments were observed on FI both at 14 and 28 d.

Footpad development and dermatitis

The histological structure of the footpad *papillae* was affected ($P<0.05$) by the sex of the chickens (Table V – 3). Both dermis length and *papillae* height were longer on females in comparison with males. No significant pairwise correlations between skin layers dimensions and BW were observed (Table V – 4). On FPD evaluation at 7 d no differences ($P>0.05$) between TEM and sex were observed on the incidence of lesions (Table V – 8 and 9). However, on the 13 d evaluation the LH TEM increased ($P<0.05$) the severity paw of lesions of the chickens. When evaluating FPD at 28 d no effect ($P>0.05$) of treatments were observed when litter moisture was used as covariate. Although, when litter moisture was not considered in the analysis a tendency ($P=0.087$) of TEM effect on FPD severity was observed. Again, FPD severity was increased when the chickens were exposed to LH TEM. No significant pairwise correlations between BW and histological measurements were obtained.

DISCUSSION

The egg incubation period is an important phase on chicken development and, even though sometimes it is disregarded, it can impact performance at market age (Hulet et al., 2007; Lindgren and Altimiras, 2011). The effects of incubation temperatures described by several authors on chicken BW at hatch are incongruous. Molenaar et al. (2011) described reduced yolk-free BW in an experiment where eggs were stressed with high eggshell temperatures (>38.9ºC) after 7 d of incubation. However, Hulet et al. (2007) reported heavier chickens coming from eggs incubated with high eggshell temperatures (39.7ºC) during the last 4 d of incubation in comparison with chickens incubated with middle (38.6ºC) and low (37.5ºC) eggshell temperatures at hatch. In the present experiment, when looking to the chicken sampled data, the LH TEM reduced BW with and without yolk at hatch which is in agreement with what was observed by Molenaar et al. (2011). Conversely, when looking to the hatch BW of the chickens placed in the pens, it was observed an effect in the opposite direction. When increasing the number of chickens assessed for BW it was observed that LH TEM increased BW at hatch. This BW difference might be due to higher residual yolk of the chickens stressed with LH TEM however, the yolk usage was not affected by the TEM treatments on the chickens sampled. Nevertheless, the increase BW induced by LH TEM observed in the data presented here in, fits in what was described by Hulet et al. (2007). On the BW gain evaluation during the grow-out period only females were affected by the TEM treatments at 28 d. The difference of BW gain effect of TEM between sexes might be explained by the differences on residual yolk at hatch. At hatch females had approximately 1.18% lower usage of yolk in comparison with males. On the performance parameters FCR was

negatively affected by LH TEM. The same effect of high incubation temperatures was reported by Hulet et al. (2007) at 21 d.

Based on the histological results, demonstrated a higher footpad skin dermis development was observed on females when comparing with males, and this effect might explain the high predisposition for FPD of males described by some authors (Greene et al., 1985; Bilgili et al., 2006; Nagaraj et al., 2007). Although in the present experiment no effect of sex was observed on the three FPD evaluations. Nevertheless, thickness of the skin dermis is related with skin strength (Christensen et al., 1994) mainly due to the high collagen content of this skin layer (Ramshaw et al., 1986; Christensen et al., 1994). Consequently any reduction of this layer may impact footpad skin integrity when exposed to adverse conditions. The effect of sex on *papillae* height it is probably due to the effect observed on dermis. It should be considered that the height of *papillae* was obtained by measuring the distance from the bottom of dermis layer to the top of SC.

The FPD evaluation performed at 7 d demonstrated that chickens are affected early in life by this pathological condition however, no differences between the TEM treatments were observed on the incidence of FPD by the end of the first week of life. Nevertheless, when assessing FPD severity at 13 d it was patent that chickens incubated with improper incubation conditions had an increased probability of develop severe lesions. The probability of LH chickens to develop lesions with brown discoloration or ulceration were 4.59% and 3.82% respectively higher than the S chickens. Conversely, the probability of S chickens of not having any paw lesion were 7.70% higher than the ones incubated with LH TEM. Even though litter conditions have been reported as the major etiological factor for FPD (Martland, 1984; 1985; Shepherd and Fairchild, 2010; Youssef et al., 2011; De Jong et al., 2012), no correlation of litter moisture with FPD score was observed at 13 d.

However, when evaluating FPD at 28 d this parameter had a central role on FPD severity. The average±SD of litter moisture percentage for 13 and 28 d were 44.1±7.8 and 44.4±6.6 respectively, therefore no much variation of litter moisture content was observed between the two the evaluations. Furthermore, at 28 d age the effect of TEM treatments were no longer observed which can probably be due to the overcome effect litter moisture over the TEM treatments. When litter moisture was not controlled as covariate on the statistical analysis a trend (P=0.087) of incubation TEM was still observed. Again LH TEM chickens had worst FPD scores when comparing with S chickens. It was observed that when litter moisture was not controlled the probability of LH chickens to have paws with brown discoloration and ulceration was 3.26% and 3.85% respectively higher than S chickens. An interesting parameter to look at is the different response between birds incubated with the two TEM treatments to litter moisture. By the linear regression models (Figure V - 1) it was observed that for each litter moisture percentual increment the worsening on FPD score was higher in LH chickens when comparing with S chickens. The test of differences between slopes indicated a trend (P=0.13) on differences between the two TEM. Chickens incubated with LH TEM when exposed to 1% extra of litter moisture had an increase of 0.04 of FPD score. When looking to FPD scores of S chickens the FPD scores vary around half (0.02) of the LH chickens when increasing one unit of litter moisture. The minimum and maximum litter moisture values observed at 28 d were 32.5% and 57.8% respectively.

Considering the results obtained in this experiment it is plausible that the differences of FPD incidence between hatcheries observed by De Jong et al, (2012) might be due to differences in incubation temperature management. Furthermore, it was demonstrated that incubation temperatures affect FPD incidence early in life and increase the bird

susceptibility to litter moisture four wk of age. Furthermore, the detrimental effect of TEM on FCR has to be considered since it can impact company revenues at the end of the production cycle.

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Table V – 1. Ingredient composition (%) and formulated nutrient contents of the starter and grower diets

Ingredients	Starter (1 - 14d)	Grower (15 - 28d)
	---------- % ----------	
Corn	52.13	58.71
Soybean meal, 48%	32.59	26.89
Distillers dried grains with solubles	6.00	6.00
Poultry fat	3.50	3.03
Poultry by-product meal	2.00	2.00
Salt (NaCl)	0.322	0.252
Limestone	1.44	1.22
Dicalcium phosphate, 18.5%	0.75	0.52
DL-methionine	0.27	0.26
L-lysine-HCl, 78,8%	0.16	0.21
Choline chloride, 60%	0.20	0.20
Sodium bicarbonate	0.26	0.30
L-threonine, 98%	0.02	0.05
Coccidiostat[1]	0.05	0.05
Mineral premix[2]	0.20	0.20
Vitamin premix[3]	0.10	0.10
Phytase[4]	0.008	0.008
Total	100.00	100.00
Nutrient composition		
ME, kcal/kg	3,050	3,100
CP, %	22.85	20.66
Calcium, %	0.92	0.78
Total phosphorus, %	0.60	0.53
Available phosphorus, %	0.34	0.29
Digestible. lysine, %	1.20	1.10
Digestible total sulfur amino acids, %	0.89	0.82
Digestible threonine, %	0.77	0.72
Digestible tryptophan, %	0.23	0.20
Sodium, %	0.24	0.23
Potassium, %	0.94	0.84
Chloride, %	0.30	0.27
Dietary electrolyte balance, mEq/100 g	277	257

[1]Coban 90® 45 (Monesin Sodium), Elanco Animal Health, Greenfield, IN

[2]The trace mineral premix supplied the following per kilogram of diet: 120 ppm of Zn as Zinc Sulfate, 10 ppm of Cu as Copper Sulfate, 120 ppm of Mn as Manganese Sulfate, 80 ppm of Fe as Iron Sulfate, 2.5 ppm of I as Calcium Iodate, 1 ppm Co as Cobalt Sulfate.

[3]Vitamins from premix provided per kilogram of premix: vitamin A, 18,739,292 IU; vitamin D_3, 6,613,868 IU; vitamin E, 66,139 IU; vitamin B_{12}, 33 mg; riboflavin, 22,046 mg; niacin, 88,185 mg; d-pantothenic acid, 30,865 mg; menadione, 3,968 mg; folic acid, 2,646 mg; vitamin B_6, 7,716 mg; thiamine, 5,512 mg; biotin, 176 mg.

[4]Quantum Blue 500 FTU/kg (AB Vista Feed Ingredients, Marlborough, UK).

Table V – 2. Effect of incubation temperature profiles and sex on BW with and without yolk and residual yolk retention at hatch

Incubation temperature	Sex	BW (g)		Residual yolk (%)
		With yolk	Without yolk	
Standard		42.7[a]	38.8[a]	9.21
Low – high		40.1[b]	36.9[b]	9.43
	Male	41.8	38.1	8.73
	Female	41.0	37.5	9.91
Standard	Male	42.8	38.8	9.48
	Female	42.6	38.9	8.93
Low – high	Male	40.8	37.5	8.52
	Female	39.4	36.0	10.34
Pooled SEM		2.8	2.4	1.8
Source of variation		---------- *P*–values ----------		
Incubation temperature		0.009	0.016	0.772
Sex		0.362	0.408	0.058
Incubation temperature x sex		0.534	0.320	0.285

[1]Values are means ± SEM of 20 broiler chickens per treatment

[a,b] Means in a column not sharing a common superscript are significantly different ($P \leq 0.05$) by Student's t test

Table V – 3. Effect of incubation temperature profiles and sex on *stratus corneum*, epidermis and dermis development and *papillae* structure at hatch

Incubation temperature	Sex	*Stratus corneum*		Epidermis		Dermis		*Papillae*	
		Length	Area	Length	Area	Length	Area	Height	Width
		(µm)	(µm^2×10^3)	(µm)	(µm^2×10^3)	(µm)	(µm^2×10^3)	--- (µm)---	
Standard		40	17.9	78	30.0	206	44.1	338	310
Low - high		36	16.6	73	29.4	213	46.8	322	303
	Male	36	15.5	70	27.4	191[b]	43.3	298[b]	303
	Female	40	19.0	81	32.0	227[a]	47.6	362[a]	311
Standard	Male	38	16.9	77	29.7	185	43.7	300	313
Standard	Female	42	18.8	79	30.3	226	44.4	377	306
Low - high	Male	34	14.2	63	25.1	198	42.9	295	294
Low - high	Female	37	19.1	82	33.7	229	50.8	348	315
Pooled SEM		7	5.1	19	9.3	40	8.0	46	32
Source of variation					*P*–values				
Incubation Temperature		0.127	0.526	0.450	0.851	0.635	0.368	0.346	0.683
Sex		0.162	0.082	0.154	0.199	0.023	0.170	0.001	0.554
IT x S		0.802	0.433	0.253	0.264	0.735	0.239	0.502	0.270

[1]Values are means of 10 chickens Cobb 500
[2] IT – Incubation temperature
[3] S – Sex
[a,b] Means in a column not sharing a common superscript are significantly different (P<0.05) by Student's t or Tukey's test

Table V - 4. Pairwise correlations between BW and residual yolk with histological measurements at hatch

Variable correlation		BW	Residual yolk
		------ *r* ------	
Lenght	*Stratus corneum*	-0.06	-0.36
	Epidermis	-0.09	-0.35
	Dermis	-0.31	-0.26
Area	*Stratus corneum*	-0.21	-0.11
	Epidermis	-0.20	-0.11
	Dermis	-0.19	0.001
Height		-0.27	-0.29
Width		-0.17	-0.56

* Significant at $\alpha<0.05$ level

Table V – 5. Effect of incubation temperature profiles on female, male and group chicken BW at hatch, 14 and 28d of age

Incubation temperature	BW (g)								
	0 d			14 d			28 d		
	Female	Male	Group	Female	Male	Group	Female	Male	Group
Standard	41.03[b]	41.74[b]	41.59[b]	511	530	517	1614	1812	1717
Low - high	43.20[a]	43.38[a]	43.29[a]	510	532	516	1585	1830	1705
Pooled SEM	1.26	1.49	1.21	15	18	19	53	61	46
Source of variation	---------- *P*–values ----------								
Incubation profile	<0.001	0.007	<0.001	0.894	0.793	0.857	0.177	0.463	0.474

[1]Values are means of 15 pens per incubation temperature treatment with 6 female and 6 male chickens for 14 d, and 5 female and 5 male for 28 d

[a,b] Means in a column not sharing a common superscript are significantly different ($P<0.05$) by Student's t test

Table V – 6. Effect of incubation temperature profiles on female, male and group chicken BW gain (BWG) at hatch, 14 and 28d of age

Incubation temperature	BWG (g)					
	0-14 d			0-28 d		
	Female	Male	Group	Female	Male	Group
Standard	468	489	475	1573[a]	1750	1676
Low - high	467	486	471	1531[b]	1784	1661
Pooled SEM	15	18	19	46	50	45
Source of variation	---------- *P*–values ----------					
Incubation profile	0.762	0.777	0.658	0.034	0.093	0.410

[1]Values are means of 15 pens per incubation temperature treatment with 6 female and 6 male chickens for 14 d, and 5 female and 5 male for 28 d

[a,b] Means in a column not sharing a common superscript are significantly different ($P<0.05$) by Student's t test

Table V – 7. Effect of incubation temperature profiles on feed intake (FI), and feed conversion ratio (FCR) at 14 and 28d of age

Incubation temperature	FI (g)		FCR (g:g)	
	0-14d	0-28d	0-14d	0-28d
Standard	603	2452	1.27	1.49[b]
Low - high	590	2522	1.25	1.52[a]
Pooled SEM	18	109	0.04	0.04
Source of variation	---------- *P*–values ----------			
Incubation profile	0.065	0.113	0.266	0.053

[1]Values are means of 15 pens per incubation temperature treatment with 6 female and 6 male chickens for 14 d, and 5 female and 5 male for 28 d

[a,b] Means in a column not sharing a common superscript are significantly different ($P \leq 0.05$) by Student's t test

Table V – 8. Effect of incubation temperature profiles and sex on footpad dermatitis (FPD) severity score at 7, 13 and 28 d of age

Incubation temperature	Sex	FPD score (0 to4)			
		7d	13d	28d	
				Considering litter moisture	Not considering litter moisture
Standard		0.34	1.06[b]	1.38	1.35
Low - high		0.41	1.33[a]	1.59	1.61
	Female	0.34	1.20	1.47	1.47
	Male	0.41	1.06	1.48	1.48
Standard	Female	0.31	1.03	1.37	1.34
Standard	Male	0.37	1.09	1.39	1.37
Low - high	Female	0.38	1.40	1.59	1.61
Low - high	Male	0.46	1.27	1.59	1.60
Pooled SEM		0.49	0.91	0.75	0.75
Source of variation		---------- *P*–values ----------			
Incubation temperature		0.261	0.029	0.157	0.087
Sex		0.311	0.813	0.936	0.964
Incubation temperature x Sex		0.947	0.439	0.923	0.898
Litter moisture		-	0.992	0.028	-

[1]Values are means of 180 chickens per incubation temperature treatment with 90 female and 90 male chickens for 7 and 13 d, and 150 chickens per incubation temperature treatment with 75 female and 75 male chickens 28 d

[a,b] Means in a column not sharing a common superscript are significantly different ($P<0.05$) by Student's t test

Table V – 9. Effect of incubation temperature profiles and Sex on the probability of footpad dermatitis (FPD) scores incidence at 7, 13 and 28 d of age

IT	Sex	Probability of FPD score													
		7d		13d				28d							
								Considering litter moisture				Not considering litter moisture			
		Without	With	0	1	2	3	0	1	2	3	0	1	2	3
								%							
S		74.76	25.24	35.46	37.59	19.92	7.04	26.59	36.62	25.22	11.58	27.14	36.75	24.88	11.23
LH		70.69	29.31	27.76	36.88	24.51	10.86	22.15	35.17	27.91	14.77	21.77	35.00	28.14	15.08
	F	74.48	25.52	31.16	37.41	22.45	8.99	24.44	36.01	26.52	13.02	24.46	36.01	26.51	13.01
	M	71.00	29.00	31.94	37.48	21.98	8.60	24.20	35.93	26.67	13.20	24.32	35.97	26.59	13.11
S	F	76.28	23.72	36.38	37.56	19.39	6.67	26.86	36.69	25.05	11.40	27.41	36.81	24.72	11.06
S	M	73.18	26.82	34.55	37.59	20.45	7.42	26.31	36.55	25.39	11.75	26.87	36.69	25.05	11.40
LH	F	72.59	27.41	26.15	36.51	25.48	11.86	22.13	35.16	27.93	14.79	21.66	34.96	28.21	15.18
LH	M	68.72	31.28	29.39	37.18	23.52	9.92	22.18	35.18	27.90	14.75	21.89	35.06	28.07	14.98

Source of variation	---------- *P*–values ----------			
IT	0.261	0.029	0.157	0.087
S	0.311	0.813	0.936	0.964
IT x S	0.947	0.439	0.923	0.898
Litter moisture	-	0.992	0.028	-

[1] S – Standard
[2] LH – Low-high
[3] F – Female
[4] M – Male
[5] IT – Incubation temperature
[6] S – Sex

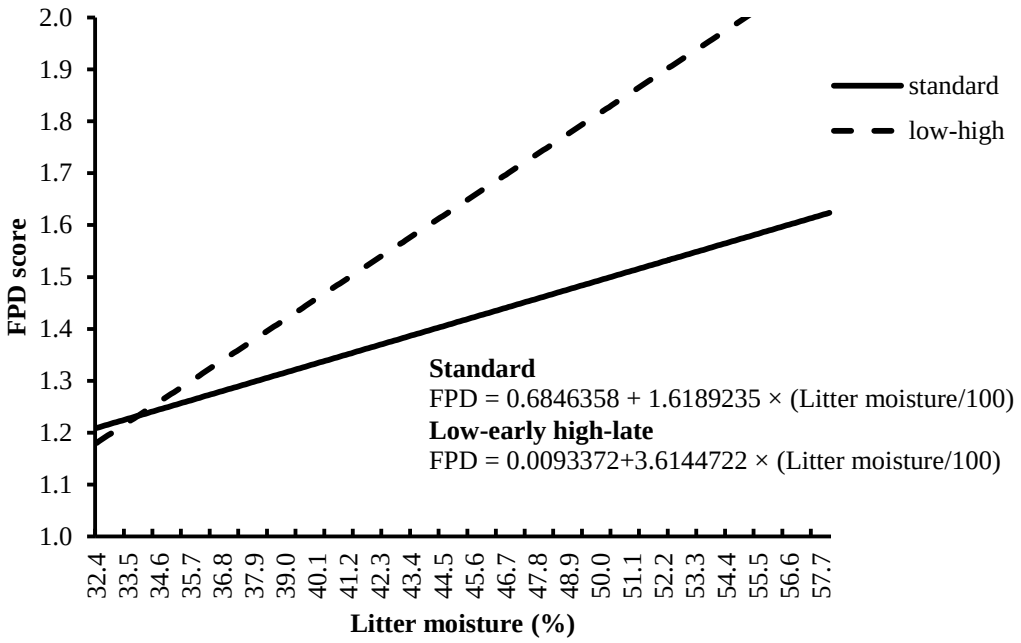

Figure V – 1. Effect of litter moisture on footpad dermatitis (FPD) severity score between chickens incubated with standard and low-early high-late incubation temperature profiles at 28 d of age

CONCLUSIONS

Considering the increasing importance of footpad dermatitis (**FPD**) for the poultry industry, mainly related with animal welfare and company profitability, it is essential to understand all the factors that cause pathological changes in the paw. The etiology of FPD lesions is believed to be multifactorial, though litter management and bird nutrition have been considered the leading factors on FPD onset. However, within a flock that is exposed to the same litter conditions and nutrition, it is usually observed birds with severe lesions and others with paws with no morphological changes. Therefore it might exist some factors that influence bird individual susceptibility to develop paw lesions. Therefore the objective of this project was to evaluate how factors such as incubation, parent stock management and nutrition could impact footpad skin and FPD development.

As a first approach (Chapter II), a study testing the effect on footpad structure and FPD by two incubation temperature profiles (**TEM**), normal vs. elevated, with two eggshell conductances, normal vs. reduced, was conducted in ducks. No effects on BW at hatch and 35 d were observed. Nevertheless, footpad structure was affected by the treatments at both ages. At hatch, dermis layer was affected by treatment interaction. Dermis of ducks incubated with normal TEM was negatively affected by hypoxia conditions. Additionally, at 35 days *papillae* structure was affected. Normal TEM increased the width of the *papillae* at the basis and decreased the width on the top of the *papillae* when comparing with elevated TEM. Furthermore, a tendency for the elevated TEM and reduced G to increase the probability of FPD severity lesions was observed.

In chapter III, the effect on footpad skin development of two breeder feed restriction programs, skip-a-day feeding vs. every-day feeding, and two TEM, standard (**S**) vs, low-

early high-late (**LH**), were tested. Performance of birds incubated under LH TEM was negatively affected at 7 and 21 d. The footpad skin structure was affected by the treatments at hatch, 7 and 22 d. Skip-a-day progeny showed to be more susceptible to TEM presenting different development of *startus corneum* and epidermis between the two TEM treatments. Furthermore, dermis parameters were negatively affected by LH TEM both at 7 and 22 d, suggesting that this TEM can impact footpad skin strength. It was demonstrated that parent stock management affects embryo susceptibility to incubation conditions and incubations conditions may affect negatively skin development.

On chapter IV, the same TEM, S vs. LH, with two dietary trace mineral sources, inorganic vs. organic, with reduction of inclusion levels of the organic sources, were used as treatments. The treatments intend to determine the effects on footpad skin development and collagen content. At 21 d BW and FCR were enhanced by S TEM. Footpad skin dermis length and area were increased by S TEM at hatch. Additionally, the organic trace minerals diets increased dermis parameters at 21 d. However, when measuring collagen content no differences between the treatments were observed. Therefore, based on the data of the present experiment, there is potential for trace mineral reduction when using organic sources and that stressful incubation temperatures have an effect on skin development during embryogenesis.

The chapter V, aimed to test and validate if the changes induced by TEM treatments on footpad skin structure observed on chapters III and IV had any impact on FPD severity when the birds were housed under simulated commercially conditions. Considering this, birds incubated under S or LH treatments were evaluated for FPD at 7, 13 and 28 d of age. Contrarily with what was observed on the previous experiments, no effects of TEM were observed on footpad structure at hatch. However, when evaluating FPD severity at

13 d, birds incubated with LH TEM had increased probability to develop severe lesions. At 28 d, litter moisture became the factor that impacted the most FPD with the effect of TEM not observed. Yet, an interesting parameter observed was that LH TEM chickens developed more severe lesions when litter moisture increased than the chickens incubated with S TEM.

In summary, it was demonstrated that incubation environment, specifically incubation temperatures, affect footpad skin development and bird performance. It was shown that stressful incubation temperatures affect dermis development which may impact footpad skin strength and FPD development. Furthermore, on the first and fourth experiment more extreme incubation temperatures had a negative effect on the probability of developing severe footpad lesions at some point in the life of the birds. Additionally it was observed that this higher probability of developing lesions, by the birds incubated with extreme incubation temperatures, might be related to a higher susceptibility to the detrimental litter moisture effects on paw quality. In conclusion, parameters that affect birds individually as incubation conditions and parent stock management do have an effect on footpad skin development and can make birds more vulnerable to develop footpad lesions.