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Lameness Associated with a Possible Neural Problem in the Lower Spinal Cord of Chickens, Gallus Gallus

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LAMENESS ASSOCIATED WITH A POSSIBLE NEURAL PROBLEM IN THE LOWER SPINAL CORD OF CHICKENS, *GALLUS GALLUS*.

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ABSTRACT

Since the second half of the twentieth century lameness in broiler chickens has been known to be caused by different types of disorders, however the etiology of several type of lameness remain unknown. Because of the intense selection of broilers for rapid growth, some birds are more prone to physiological insults resulting in lameness. This study focuses on possible neural problems in the lower spinal cord associated with lameness in broilers. Broilers were raised in pens with wire floors and provided with food and water ad libitum. Three groups of birds 1) birds displaying a normal gait (Controls), 2) lame birds with normal leg bones (Neural Associated Lameness), and 3) lame birds with femoral head separation/necrosis (Bone Associated Lameness) were selected based upon behavioral observations of gait. Motor neurons in the lateral motor column of the lumbosacral region of birds in the neural associated lame group were observed to have changes in the perikarya of motor neurons. The lumbosacral segment 4 (LS 4) of the spinal cord was selected for analysis, based upon its central location in the lumbosacral region and the highest number of motor neurons when compared to other segments. In the neural associated lame group the motor neurons were more globular in appearance with a 25% to 40% reduction in protrusions from the perikarya when compared to controls. The corticosterone level, an indicator of stress, was increased 3-4 fold in the lame birds when compared to the controls. Additionally, an association between decreased percentage body weight gain and increased corticosterone level in lame birds was observed. Results of this study indicate an association between the reductions of neurites in the motor neurons of the lumbosacral spinal cord and a leg weakness in broilers with normal leg bones.

Keywords: lameness, spinal cord, lateral motor column, globular neurons, corticosterone, gait score.

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DEDICATION

I dedicate this thesis to my mother Dr. Ushadevi Nagarajan and

to my father Mr. Nagarajan Gurusamy.

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INTRODUCTION

Broilers are meat type chickens specifically produced for human consumption. Genetic selections and efficient feed conversion programs played key roles in producing the modern day broilers for fast growth. In the early 1950s, it took an average of 14 weeks to grow a broiler to a market weight of two kilograms. Today, it takes an average of 6 ½ weeks to grow a broiler to the same market weight. This resulted in an increased pressure on the legs to bear the large body weight. High body weight gain prior to maturation of the skeleton and nervous system resulted in lameness or leg weakness in some broilers, specifically, lameness occurs in 1-2% of broilers. Increased body mass places a physical stress on the developing leg bones increasing their susceptibility to bacterial infections. Lameness associated with leg bones, particularly proximal portions of the femur and tibia, is observed in most cases of lame birds, however, the cause of a small percentage of lameness in broilers with normal leg bones remains unknown. The latter mentioned lameness in broilers may be caused by an abnormality of the nervous system. Infectious diseases like avian encephalomyelitis and Newcastle Disease are known to affect the nervous system in broilers resulting in lameness. A non-infectious disease such as polyneuropathy, which affects the peripheral nervous system, is known to cause lameness in Leghorns, chickens selected for table egg production. Hence, a study was initiated in broilers to understand the relationship between the hind limb nervous system and lameness, for possible neural changes in the lower spinal cord or lumbosacral spinal cord of the lame birds with normal leg bones.

CHAPTER I

Review of Literature

1. Lameness in broiler chickens

1.1 Growth rate and increased body weight

Consumption of chicken as a form of meat has been significantly increased in the late twentieth century. Over the last 50 years, the poultry industry has been producing increasing number of meat chickens or broilers. Broilers have been genetically selected to gain high body weight to meet the consumers' needs. From the early 1950s one goal of the poultry industry was to produce broilers with increased body mass but also in a short period of time. To achieve this goal numerous studies have been completed especially focusing on genetic selection [1] and feed conversion programs. The latter method attained the significant rise in growth rate of broilers [2]. Changes in feed intake have been shown to be caused by intense genetic selection of broilers for increased body weight gain [3]. The high growth rate has shown to be a main factor associated with lameness in broilers [4].

1.2 Problems associated with fast growth rate and poor management

Due to rapid growth rate, the frequency of leg abnormalities has increased. Issues concerning leg health caused the culling of some birds before reaching market age resulting in economic loss [5]. Abnormalities in skeletal structure lead to a large proportion of the problems identified with leg weakness [6-7]. Genetic factors have been reported in broilers associated with skeletal abnormalities [8] and they also play a key role in causing leg weakness [9].

Increased body weight is one of the factors for the increased leg problems [9] and rapid growth rate results in inadequate bone strength. Genetic selection for breast muscle (pectoral) has

been reported to cause changes in the leg musculature in broilers [10]. Rapid remodeling of skeletal structure concurrent with fast muscle growth can eventually cause skeletal abnormalities [6] involving leg bones. Due to fast growth rate and increase in body weight in a short period of time there is instability in the leg to balance the whole body weight. This eventually results in decreased physical activity in broilers [11] and decreased feed and water intake which have an impact on their health [12]. Poor management such as a high stocking density has been shown to decrease the movement of broilers [13-14] and lameness is more pronounced in birds with little movement [15]. Due to increased body weight gain, the incidence of lameness is more prevalent with the increase in age.

1.3 Animal Welfare issue and stress in broilers

High body weight gain in broilers to meet consumer demands has resulted in a lack of focus on factors involved in normal locomotion, i.e. improving leg capacity to support the increased yield in body mass [6]. Intense selection for muscles has affected the development of skeletal bone structure and reproductive performance [16]. Twenty years ago an estimated report has shown an annual loss of more than $100 million to the poultry industry due to skeletal abnormalities [17]. Factors such as genetic selection, stocking density, poor litter management, light programs [18], temperature and humidity are known to cause lameness, mortality, poor leg development, lesions in hocks and foot pads, thereby raising questions of humane management for broilers [19].

Some studies have provided valuable information in addressing welfare issues. Increasing the locomotion ability would help to reduce the prevalence of lameness and it has been shown in several studies that increase in the activity would result in decreased leg associated problems in broilers [9, 11, 20-21]. For example in one study a threefold increase in the movement was observed in broilers by increasing the distance between feed and water [21], thereby improving

the activity of broilers. Leg health has also been shown to improve by slowing the growth rate and increasing the activity of broilers at the first two weeks of age [22].

Stress is an important factor in evaluating animal welfare issues, as the pain associated with leg weakness in broilers raises serious ethical concerns [23]. A mean of testing whether birds are in pain is to provide self-selection of diets. For example broilers that had leg problems prefer feed that had an analgesic drug, such as Carprofen, added to it [24-25]. One method of assessing pain behaviorally is when broilers prefer to remain in a lying state or immobile state [11, 24, 26]. Another method of detecting leg discomfort behaviorally is when the birds stretch their legs. Leg stretching behavior is found to be abnormal in broilers and it is suggested to alleviate pain due to leg weakness [15]. Thus, it is important to ease the pain in broilers and one solution is to reduce lameness. The incidence of lameness can be decreased by increasing walking ability [21], rich rearing environment [27] and effective light programs [28-29].

2. Leg disorders - A major concern in poultry industry

Body weight plays a key role in the prevalence of leg problems in broilers [30]. Several disorders have been identified due to fast growth rate. Different studies had shown the differences in the cause of leg disorders in broilers. A study in Denmark had shown that the prevalence of leg weakness in commercial broilers was particularly due to tibial dyschondroplasia (TD), varus/valgus deformations, crooked toes and foot pad burns [30]. Broilers in Australia are prone to twisted legs, focal osteodystrophy, bacterial osteomyelitis, plantar pododermitis, spondylolisthesis, and deviated toes [31]. In chickens, leg disorders can be categorized into the bone, nutritional imbalance, pathological and neurological disorders. Some of the bone disorders are caused by deficiencies in vitamins and minerals, conditions which can be mainly categorized under nutritional imbalance [32-33] and other bone disorders can be

caused by infectious agents such as *Staphylococcus aureus*, which is mainly a pathological problem. Since the nutritional imbalance and pathological agents causes leg bone problems resulting in leg weakness, both infectious and non-infectious types of bone disorders are discussed and also leg weakness caused by neurological disorders are discussed below.

2.1 Bone disorders

There are various types of bone disorders. They are described as follows.

Tibial Dyschondroplasia (TD) - Tibial dyschondroplasia can be characterized by a thick cartilaginous plug due to avascularization of cartilage in the proximal part of the tibial bone [34-35]. Diets rich in phosphorus and deficient in calcium have been shown to induce TD as early as 2 weeks of age [36]. Variations in the occurrence of TD have been observed in different genetic lines of broilers [37].

Femoral Head Necrosis (FHN) – Femoral had necrosis is characterized by degeneration of the femur head or fracture between the growth plate and the epiphysis/metaphysis [38]. The cause of femoral head necrosis is still under debate, but it can be due to infectious [31, 38] or non-infectious pathology [5]. It has been reported that femoral head necrosis can be due to growth plate cartilage and bone degeneration, necrosis and degeneration of dyschondroplastic cartilage and osteomyelitis [39]. Femoral head necrosis could be an age related factor, since degenerative bone lesions increased with age [11]. It was reported that the occurrence of lesions in the femoral head were more prevalent after 5 weeks of age [11].

Rickets – Rickets is characterized by the lack of mineralization of the growth plate resulting in more supple long bones, leading to lameness in young broilers [40]. Rickets occurs due to deficiencies of vitamin D, and inappropriate calcium/phosphorus ratios in the diet [41].

Osteochondrosis – Osteochondrosis occurs due to endochondral ossification insult in the growth plate of vertebrae, femur, tibia, tarsometatarsus and occurs in the proximal and the distal portion of bones [42]

Twisted Leg – Twisted leg in broilers can be characterized by lateral twisting of the shank region of the legs, caused by the curvature of the distal part of tibia and proximal part of metatarsus [31]. It has been reported that twisted leg is an effect of leg strain [43] and damage to the growth plate or the hock joint ligaments surrounding the joint during early growth period may lead to twisted leg [44].

Kinky back (Spondylolisthesis) – Kinky back occurs due to abnormality in the 6^{th} thoracic vertebrae resulting in compression of the spinal cord and interference with the transfer of sensory and motor information passing through that deformed area. The result is leg weakness in both legs [45-46].

Osteoporosis (Cage Layer Fatigue) – Cage layer fatigue is a frequent metabolic disorder in laying hens that are caged. Fracture of the thoracic vertebral bone results in compression/damage of the spinal cord [47], affecting both legs may result in birds walking backwards [31]. Deficiency of phosphorus during initial development of medullary bone [48-49] and calcium deficient diet results in decreased bone mineral content [49].

Osteomyelitis – Osteomyelitis is characterized in chickens showing difficulties in gait such as limping and immobility. It is caused by *Staphylococcus aureus* [31]. Bumble foot (a skin disorder) is characterized by swelling and ulcer formation on foot pads and can be caused by *Staphylococcus* and *Escherichia coli* and is considered as a combination of osteomyelitis and synovitis [31].

Tibial Head Necrosis **-** Bacterial chondronecrosis with osteomyelitis can cause degenerative lesion of the proximal part of the tibialtarsus. It can be caused by infections of Staphylococcus aureus [50], however, other bacterial species may be involved.

2.2 Neurological disorders

Lameness due to neurological disorders in chickens can be caused by infectious and non-infectious agents. Several nutritional factors are also involved in neurological disorders. Deficiencies of vitamin-E, riboflavin, and thiamine can affect central nervous system function which results in lameness [31].

Marek's Disease (MD) **–** Marek's disease in Leghorns can be characterized by paralysis and inflammation of the sciatic nerve, caused by paramyxovirus 1 virus affecting the peripheral nervous system. It can be controlled through appropriate vaccination [51-52].

Avian Encephalomyelitis (AE) **–** Avian encephalomyelitis in chickens is characterized by tremor of the head and neck with a lack of muscle coordination [53]. Avian encephalomyelitis is caused by Avian Encephalomyelitis Virus (AEV). Lesions are characterized by accumulation of neuroglia surrounding the perivascular region and neuronal degeneration [53]. It can also be controlled through vaccination [54] and it has been widely observed in Leghorns.

Newcastle Disease (ND) **–** Newcastle disease in Leghorns is characterized by paresis and paralysis [55] with lesions occurring in the central nervous system caused by neuronal necrosis, axonal degeneration, infiltration of microglia, inflammatory response through T-lymphocytes and astrogliosis [56]. Infectious lesions of Newcastle disease is caused by Newcastle Disease Virus (NDV).

Peripheral Neuropathy (PN) – Peripheral neuropathy is an autoimmune disorder characterized by paralysis and inflammation of peripheral nerves in Leghorn chickens [57-58]. It is often misdiagnosed as Marek's disease.

Type-C Botulism – Botulism can be characterized by the paralysis caused by Clostridium botulinum type-c. It has been reported in several studies, although it is rarely observed in broilers [59-60].

Although Marek's Disease, avian encephalomyelitis and Newcastle Disease have been reported in Leghorns, to prevent their occurrence current day broilers are vaccinated against these diseases. Most of the neurological disorders are known to occur in Leghorns and affect their motor systems which eventually result in paralysis, however, there are reports of leg weaknesses in broilers whose pathogenesis remains unknown [61]. Therefore, knowledge about the mechanism involved in the motor function and gross and microscopic examinations of the spinal cord would be beneficial to understand the pathogenesis of several undefined neurological disorders causing lameness in broilers.

3. Central nervous system involved in locomotion

3.1 Lumbosacral region of the spinal cord – Avian species

The Lumbosacral (LS) region of the spinal cord in birds comprises the last 3 lumbar segments and 5 sacral segments with the nomenclature LS1-LS8 (Fig. 1). Fourteen vertebrae are present in the lumbosacral region of chickens, which are fused soon after hatching, into a single bone called the synsacrum [62]. The synsacrum is unique to avian species. Within the midline region of the synsacrum is the spinal cord. Near the middle of lumbosacral region of the spinal cord an enlargement can be seen, due to the presence of the glycogen body, which has glycogen

cells. This structure is also unique to birds. Each segment of the lumbosacral region of a spinal cord has a pair of spinal nerves, each of which has a dorsal root and a ventral root.

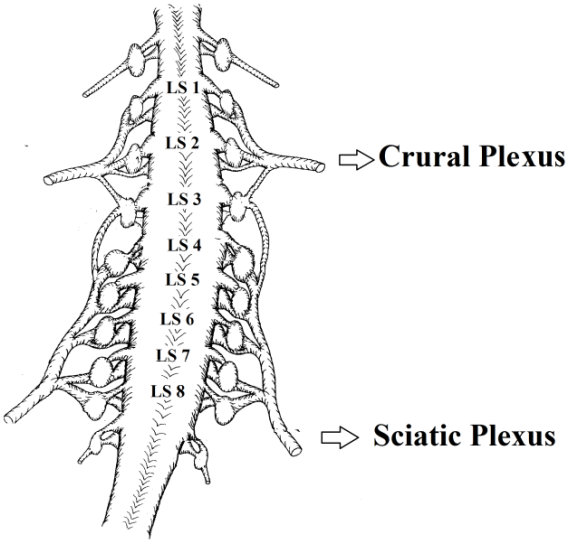

Fig. 1. Schematic diagram of the lumbosacral spinal cord of chickens. The crural plexus is formed from the roots of the first three segments (LS 1-LS 3) of the lumbosacral spinal cord and the sciatic plexus is formed from the roots of the last five segments (LS 4-LS 8) of the lumbosacral spinal cord. Both the plexus have axonal projections that innervate the leg muscles (Modified from Hollyday et al., 1977).

A plexus is a complex bundle where several nerves travel together. In the peripheral nervous system the nerves from the bundle can split apart, redistribute and innervate different muscles. Neural plexuses in the lumbosacral spinal cord are the crural plexus and the sciatic plexus. The crural plexus is formed by three spinal nerves from the last three lumbar segments and the sciatic plexus is formed by five spinal nerves of the sacral segments. Another structure associated with this region of the spinal cord is the accessory lobe. Accessory lobes are small protrusion from the ventro-lateral side of the lumbosacral region [63] and are comprised of neurons and glial cells.

3.2 Hind Limb and Locomotion

The hind limbs of chickens are primarily involved in locomotion and are well developed. Nerves from the lumbosacral region of the spinal cord innervate muscles of the hind limbs. Walking behavior of a chicken can be divided into two different phases [64]. Stance phase and swing phase. Swing phase is further subdivided into swing flexor and swing extensor phase. A single movement of a leg comprises elevating and flexing the muscles of the leg to lift it off the ground. The bird then lowers that leg and moves its body forward until the toes touch and plant on the ground. Leg muscles of the other leg are active until the other foot touches the ground. Not all muscles are involved in specific stages of locomotion. Some muscles are highly active during the stance phase and some are highly active during the swing phase, which were studied through electromyography (Table 1).

Table 1. Hind limb muscles of chickens, *Gallus gallus.*

Adductor (Puboischio femoralis) [a]	Sartorius [b]
Caudilioflexorius	Tibialis anterior [b]
Femorotibialis [b]	Accessorius
Iliofibularis [b]	Flexor hallicus longus
Iliotibialis anterior [b]	Extensor digitorum longus [b]
Iliotibialis posterior [a]	Flexor digitorum longus
Iliotrochantericus anterior and posterior [a,b]	Flexor perforans et perforatus digit II
Ischiofemoralis [a]	Flexor perforans et perforatus digit III
Ischioflexorius	Flexor perforans digiti III
Lateral gastrocnemius [a]	Flexor hallicus brevis
Medial gastrocnemius [a]	Extensor hallicus brevis
Peroneus longus	Extensor digitorum brevis

a - muscles highly active in stance phase; b - muscles highly active in swing phase

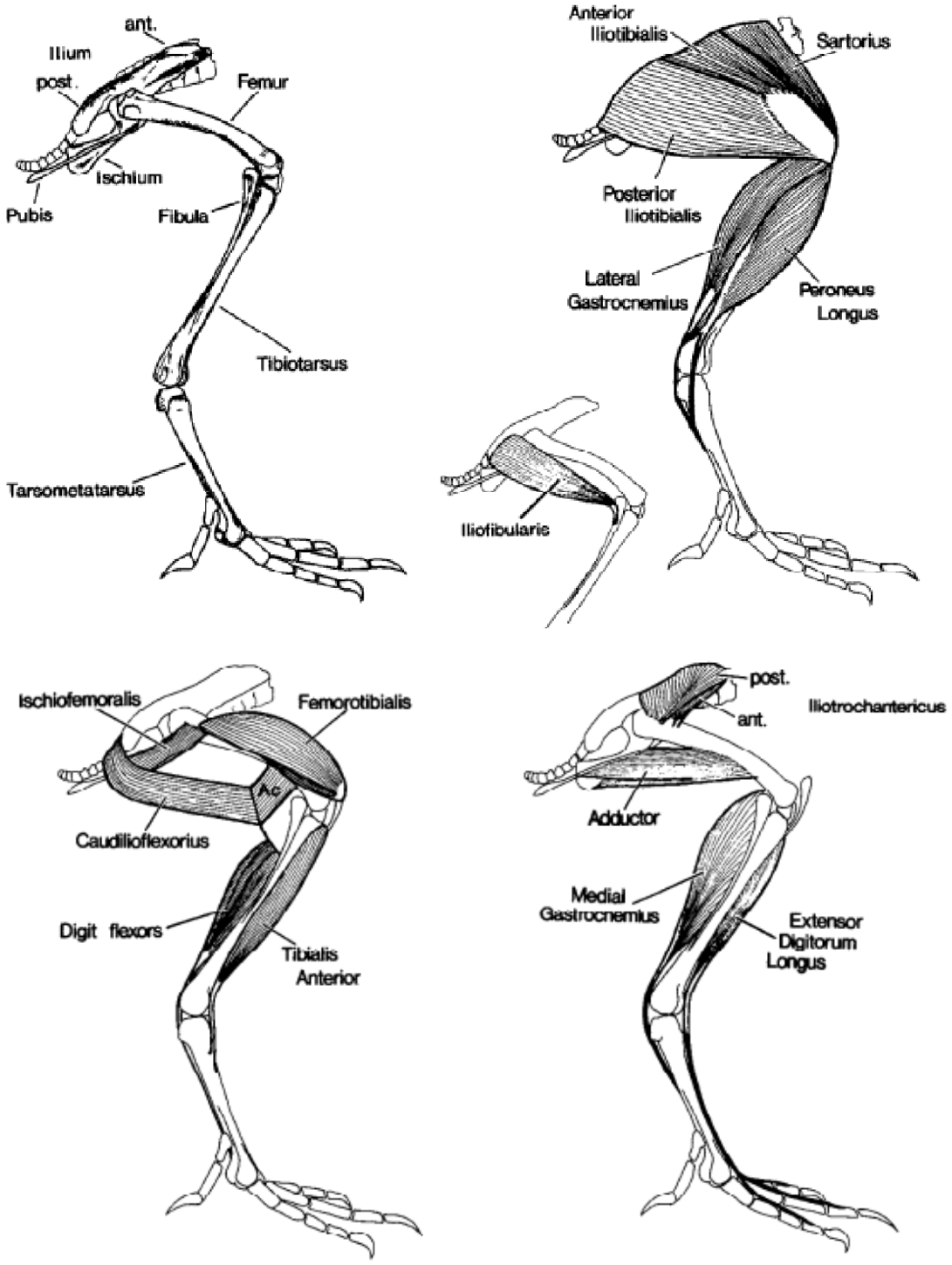

Fig. 2. Muscles highly active during the stance and the swing phase (Table 1) in the walking behavior of the chickens, Gallus gallus (from Jacobson et al., 1982).

3.3 Sensory and motor system involved in gait

After the plexus the spinal nerves separate and innervate muscles for motor function or skin for sensory function. A dorsal root ganglion is present at the sides of each segment of the spinal cord which houses the perikarya of sensory neurons involved in relaying sensory information from the periphery to the central nervous system. Motor neurons, found in the lateral motor column or lamina 9 (Fig. 4), have axonal projections to different muscles in the leg. The sensory axons from the muscles and motor axons to the muscles travel together except in the plexus [65] indicating the importance of neural activity, to and from specific muscle, housed in the same lumbosacral segment of the spinal cord. A region near the dorsal horn of the spinal cord is important in relaying nociception. It is an important region in the spinal cord in assessing pain in chickens associated with lameness. Nociception can be studied through markers such as substance P and calbindin D 28K molecules [66-67].

The leg motor system plays a key role in locomotion. As described in the bone disorders section above, any abnormality in the vertebral bones due to deficiencies in nutrition or a pathological cause could result in compression of the spinal cord. Consequently, motor neurons in the spinal segments caudal to the affected region may not get proper neural input from the brain leading to alterations in function of the motor neurons resulting in movement disorders. Thus the motor system and the sensory system play key roles in the gait of birds.

3.4 Organization of motor neurons in avians and mammals

Motor neurons involved in leg movement are confined to specific regions in the spinal cord. In the lumbosacral spinal cord, the laminar organization extends from the dorsal horn to the ventro-lateral side of gray matter (Fig. 4). Motor neurons are present only in laminae 9 of the spinal cord, which is more commonly known as the lateral motor column (LMC).

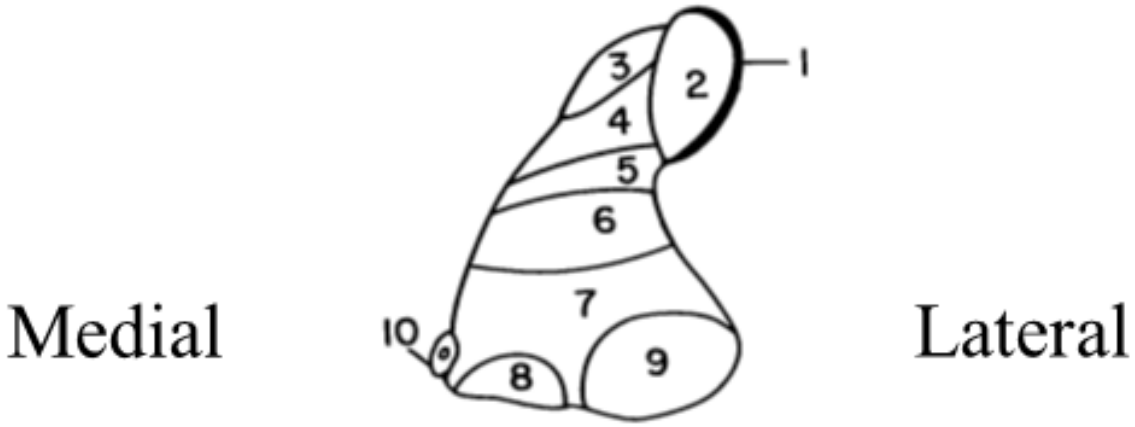

Fig. 4. A Laminar organization in the gray matter of lumbosacral spinal cord of chicken has nine different regions, laminae 1-9. (Modified from Martin, 1979).

The organization of motor neurons in the lateral motor column of the spinal cord is quite similar in most vertebrates [68], including: rat [69], cat [70], chicken [71] and frog [72]. The motor neurons, in the lumbosacral region, projecting to specific muscles are confined to specific segments in the lumbosacral spinal cord of chickens (Fig. 5). The neural-muscular organization of motor neurons found in the spinal cord was mapped in chickens using retrograde tract-tracing methods by their axonal projections found in the muscles [71, 73-74].

Having knowledge of the organization of the motor neurons in the lumbosacral region, may aid in assessing leg weakness particularly if abnormalities in the vertebrae or nervous system of the chickens are suspected.

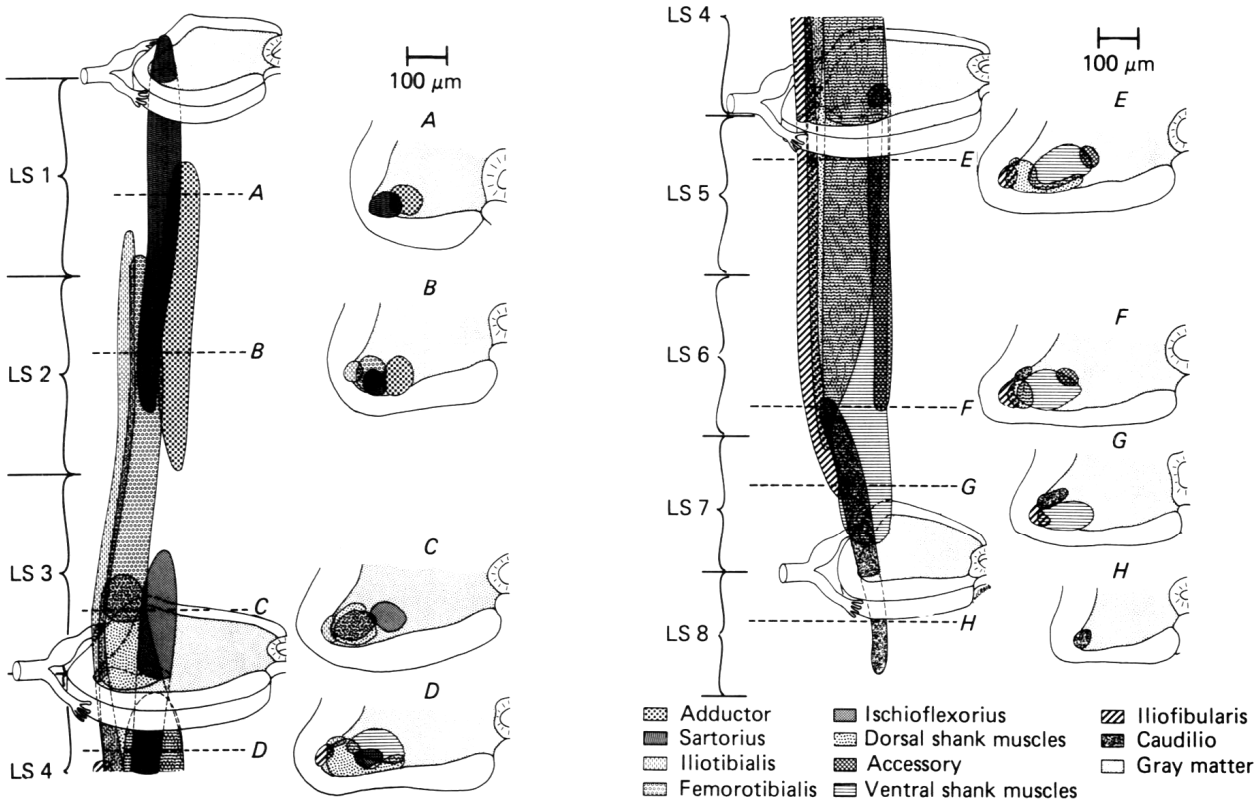

Fig. 5. Motor neuron map in the lateral motor column of each segment (LS 1-8) of the lumbosacral spinal cord of chickens. Dorsal shank muscles include extensor digitorum longus and tibialis anterior; ventral shank muscles include medial and lateral gastrocnemius (from Landmesser, 1978).

3.5 Dysfunctional motor system affecting gait

Studies have been reported about abnormalities in the spinal cord affecting gait, especially in mammals. Spinal Muscular Atrophy (SMA) is a neuromuscular genetic disorder, caused by a mutation in the Survival Motor Neuron gene (SMN) leading to degeneration of motor neurons in the spinal cord [75]. Spinal muscular atrophy causes muscle weakness and locomotion disability and it has also been reported in canines [76-77]. Amyotrophic lateral sclerosis (ALS) is a motor neuron disorder, causing motor neuron degeneration in the spinal cord, brain stem, cortico-spinal tract and primary motor cortex resulting in progressive paralysis. It affects 1-2.5 individuals in 100,000 human populations [78]. Other similar disorders which affect motor neurons are Progressive Bulbar Palsy (PBP), Progressive Muscular Atrophy (PMA), Primary Lateral

Sclerosis (PLS) and flail arm syndrome [79]. Spinal Cord Injury affects variable functions of the motor/sensory system depending upon the location of lesions in the spinal cord [80]. The degree of abnormalities affecting the spinal cord which hinders walking ability is very wide. Thus understanding deficiencies or defects in the motor system of mammals may help in understanding leg weakness in broilers.

A number of techniques have been developed to diagnose the early onset of neural disorders. One of the most powerful techniques is DNA microarray analysis which can be used during the early stages of diseases to find genes involved in causing neurological disorders [80]. Gene therapy can be used for treating genetic disorders and was shown effective in mouse models for spinal muscular atrophy [81].

3.6 Comparison of leg disorders in mammals and birds

Understanding the similarities of leg disorders between mammals and birds will provide important knowledge towards using chickens as biomedical models for human motor diseases. There have been numerous reports of leg weakness in poultry and other animals, such as pigs and dairy cattle, whose products are used for human consumption [9]. Similarities between aseptic femoral head necrosis in broilers and separated femoral head syndrome and Legg-Perthes disease in human has been reported [31, 82] where the avascularization of the proximal part of the epiphysis occurs causing a necrotic condition. The etiology of the latter is unknown [83]. Neurological disorders in cats, dogs and humans are known to severely affect gait. Polyneuropathy has been reported in White Leghorns (Acute paretic syndrome) with inflammation of peripheral nerves causing lameness [57]. Rottweiler's, one of the breeds of dog, a polyneuropathy condition has been known to affect gait [84]. In both cases demyelination and infiltration of inflammatory cells were observed in cranial and peripheral nerves. It is possible

there could be a similar kind of neurologic problem observed in broilers. Acute paretic syndrome in Leghorns [58] has been shown to have similarities to acute inflammatory demyelination polyneuropathy (AIDP) in humans. Thus, if there is a pathological relationship between polyneuropathy and a certain type of lameness in broilers, the broiler chickens may serve as a useful biomedical model for human research.

3.7 Behavioral Assessment of lameness in broilers

Behavioral assessment is very important in poultry in order to have sufficient knowledge to address animal welfare issues. Walking ability of broilers decreases as they reach market age [15]. In general, walking behavior can be studied in great detail through use of a video assisted tread mill [85-86], opto-electronic motion analysis system [87] and intra muscular electromyography [64]. In broilers, a force measurement platform system has been shown to be a reliable tool for the assessment of lame chickens, which measures the ground reaction force and femur strength during walking [11]. Latency to lie is another method to assess lameness in broilers where they are forced to stand using luke warm water and the time duration is measured for each bird to assess the degree of lameness [88]. All these behavioral assessments described above are objective methods, however, are not practical to use in huge flocks of broilers. There are however, other methods like subjective behavioral scoring systems used commercially for easy and quick ways of assessing lameness in large numbers of broilers. Kestin's gait scoring system [9], a commercial three point gait scoring system [89] and a few other scoring systems are some of the behavioral scoring system for assessing lameness in broilers.

4. Hypothesis

Based upon the information from the literatures there are neurological disorders in chickens known to cause leg weakness. Knowledge about nervous system involvement in locomotion together with behavioral assessment methods will provide clues to understand neural structures involved in gait. Provided with this information, a study was initiated (discussed in chapter II) to examine the pathogenesis of an unidentified leg weakness in broilers with normal leg bones which could be caused by a possible neural problem.

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CHAPTER II

Morphological Changes in the Lower Spinal Cord Motor Neurons and their Association with Leg Weakness in Chickens, *Gallus gallus.*

1. Introduction

Leg weakness in broiler chickens has been considered a serious problem in the poultry industry. From the early 1960s broilers were produced at a faster growth rate compared to earlier times to increase meat production in shorter periods of time. Since then the average growth rate of broilers has been considerably increased from hatch to market age [1]. Due to fast growth rate, broilers are more prone to leg disorders [2-3]. Several individual studies have shown the prevalence of leg weakness is caused by several disorders. Some of them include skeletal [4], pathological, neurological, and nutritional disorders [5]. Bone disorders such as tibial dyschondroplasia - TD [6-8], femoral head separation/necrosis – FHS/FHN [9], tibial head necrosis – THN [10], and spondylolisthesis [11] are largely responsible for causing lameness in broilers. Genetic selection has played a significant role in reducing the occurrence of leg disorders [12-13], however, lameness remains a problem in the poultry industry including animal welfare concerns [14-15] and economic losses. A number of studies have addressed bone disorders as a primary cause of lameness in poultry. Nonetheless, there are other factors such as neurological and pathological disorders involved in lameness.

Several neurological disorders such as avian encephalomyelitis [16-17], Marek's disease [18-19], and New Castle disease [20] in broilers and Leghorns, and polyneuropathy [21-22] in Leghorns are known to cause lameness. Avian encephalomyelitis, Marek's disease and New Castle disease are known to cause degeneration of neurons; thereby affecting leg health, however, occurrences of avian encephalomyelitis [23-24], and Marek's disease [25-27] have been considerably reduced through vaccinations. Peripheral neuropathy, an autoimmune disorder has been reported in recent studies, affects the peripheral nervous system thereby causing leg paralysis in Leghorns. Although infectious neurological disorders can be identified, there are

possibilities of other types of neurological disorders involved in lameness due to physical stress of the large body weight of current day broilers.

In one study [28] it was reported that there was approximately a 1.75% incidence of lameness not caused by bone disorders. Therefore, a study was initiated to determine whether or not the low incidence of lameness from an unknown cause which could be the result of a neural disorder. The lower spinal cord and its peripheral nerves were examined. In addition, a behavioral scoring system was employed to separate birds with normal gait from boilers with degrees of lameness. The morphology of the motor neurons as well as stress levels, using corticosterone as an indicator, was studied in broilers.

2. Materials and Methods

2.1 Bird Management

Broilers used in the overall study came from different lines. Chicks for each experiment were reared from hatches that were raised in a brooder until 2 weeks of age and were moved to floor pens (2.43 x 0.91 meters, length x width) with floors constructed of wire hardware cloth. Chicks were maintained at a decreasing setting of temperature (from 32º C at hatch to 24º C at 4 weeks of age and older). Broilers were provided with food and water ad libitum throughout each experiment. All procedures and experimental protocols involving animals were approved by the University of Arkansas Institutional Animal Care and Use Committee.

2.2 Behavioral assessment and experimental groups

Birds were gait scored by three different gait scoring methods during the course of the study. In initial experiments, a three point commercial gait scoring system was used (see supplementary

material) having a gait score of ‘0’ for a normal gait and scores of ‘1’ and ‘2’ for abnormal gaits, the latter meaning unable to walk.

A second method, the Kestin six point gait scoring system [29] was used in subsequent experiments to distinctly separate birds based upon their degree of lameness by increasing the number of behavioral categories [Gait score of ‘0’ (GS 0) = normal walking to gait score of ‘5’ (GS 5) = unable to stand or walk, (supplementary material)]. A runway (3.04 x 0.25 x 0.25 meters, length x width x height) made of wire hardware cloth was built and birds, one at a time, were observed carefully. All the birds were feed restricted for 1 hour to motivate them to walk through the runway to obtain the feed as well as by attraction of a conspecific bird placed at the end of the runway. A third method, five point gait scoring system (Table 1) was then developed due to the use of the runway and weekly repeated observations were recorded for each broiler.

Initially, two experimental groups were planned for each experiments, one control group showing normal walking behavior (GS=0) and the other group that was lame (GS≥2). When birds were autopsied, however, a high prevalence femoral head separation and/or necrosis in lame birds were observed. Since the goal of the experiment was to determine whether or not lameness occurred due to a neural disorder, it was critical to make sure the leg bones were normal. Three groups were then used in each experiment: (a) control group (GS = 0), (b) a lame group with normal leg bones, proposed neural (GS ≥ 2), and (c) a bone associated lame group showing FHS/FHN, THN or TD (GS ≥ 2).

2.3 Sample collection for histological staining

In an experiment, birds were euthanized using sodium pentobarbital solution (30 mg/kg) and grouped based on gait scores and bone condition after necropsy. The synsacrum of the pelvic region of the broiler chickens was collected for each bird and placed in 4% paraformaldehyde for

fixation. Each dissected lower spinal cord (lumbosacral region) was placed in the same fixative overnight, cryopreserved in 30% sucrose solution in 0.1M PBS until they sank. Samples were either embedded in a gelatin-egg albumin medium (3% gelatin in 30% egg-albumin, [30]) and/or directly sectioned coronally at 40 µm using a cryostat (Leica CM 3050 S, Leica Microsystems, Thronwood, NY). Sections were collected in 0.1 M PBS and mounted on slides. The slides, with coronal section of the lumbosacral region of the spinal cord were then stained using 0.1% Cresylecht Violet solution and then cover slipped.

2.4 Sample collection for Immunostaining

Sections of lower spinal cord were immunostained for Choline Acetyl Transferase (ChAT) using polyclonal antibody to chicken ChAT. Birds were anesthetized using sodium pentobarbital solution (30 mg/kg body weight) followed by heart perfusion with 150-200 ml of heparinized phosphate buffer saline (0.1 M PBS, 0.1% sodium nitrite, pH 7.4). Thereafter, birds were perfused with 300 ml of 4% paraformaldehyde (PFA) solution (0.1M PBS, pH 7.4). The synsacral region of the birds were collected and fixed overnight in the same fixative. The lumbosacral region of the spinal cord of each sample was carefully dissected out and discrete segments of the lumbosacral region were again returned to 4% PFA for a few hours. The segments were then placed in 30% sucrose until they sunk. The lumbosacral region 4 (LS 4) was sectioned at 40µm and placed in cryoprotective solution.

Coronal sections of the lumbosacral region of the spinal cord were transferred to phosphate buffer saline (PBS) from the cryoprotective solution, treated in 0.6% H_2O_2 in PBS for 30 minutes followed by rinses in PBS. Sections were rinsed in 0.4% Triton X-100 for permeabilization of the sections, and incubated in 5% normal goat serum for 30 min to minimize non-specific binding of the antibody. Sections were incubated in the primary ChAT antibody (kindly provided

by Dr. Miles Epstein, University of Wisconsin), at1:1000 dilution in PBS containing 1% NGS, 0.2% Triton X-100 and 0.1% Sodium azide, for at least 40 hours at 4ºC. Sections were then incubated for 90 minutes in biotinylated goat anti-rabbit antibody (Vector Laboratories, Burlingame, CA) diluted 1:500 in PBS with 0.2% Triton X-100, followed by incubation for 90 minutes with Avidin Biotinylated Horseradish Peroxidase Complex (ABC-HRP complex, Vector Laboratories) diluted 1:5 in PBS containing 0.2% Triton X-100 and bovine serum albumin. Diaminobenzidine (DAB) was used as a chromogen to visualize immunoreactivity. Sections were rinsed with PBS, distilled water, mounted on clean glass slides and cover slipped with Histomount (National Diagnostics, Atlanta, GA).

2.5 Radioimmunoassay using corticosterone

Stress levels of the sampled birds were determining by plasma corticosterone (CORT) using a radioimmunoassay (RIA) [31-32]. Blood sample was collected using heparinized syringes from the brachial vein of each bird and then transferred to borosilicate tubes. Plasma was collected from each blood sample by centrifugation and stored in -20º C until used for the assay. All the samples were assayed in duplicate. Steroids from plasma samples (200 µl) were first extracted with ethyl ether (2 ml) in borosilicate glass tubes that were vortexed for 30 minutes at room temperature. In the second extraction step the vortexed tubes were placed in methanol-dry ice bath to separate the water-soluble fractions that were then transferred to new tubes. Dried extracts were obtained by evaporation of ether in a water bath at 39ºC. The extracts were reconstituted with 400 µl of assay buffer (0.1 M PBS with 0.1% gelatin and 0.1% sodium azide, pH 7.0) and vortexed for 5 minutes and equilibrated overnight at 4ºC. The tubes were vortexed for 5 minutes after equilibration. Then 100 µl of 5% normal rabbit serum was added only to non specific binding tubes. Monoclonal primary antibody (100 µl) against corticosterone raised in

rabbit (Fitzferald Inc., Concord, MA) was added, followed by addition of 100 µl of radioactive ^{125}I Corticosterone ~20,000 CPM (MP Biomedicals Inc., Orangeburg, NY). The tubes were then vortexed and incubated for 24 hours at 4ºC. After incubation, 200 µl of secondary antibody, sheep anti-rabbit 1:40 (MP Biomedicals Inc., Orangeburg, NY) and 500 µl of 6% polyethylene glycol were added. Tubes were then vortexed for 5 minutes and incubated at 4ºC for 1 hour. Pellets were formed at the bottom of the tubes after centrifugation at 3000 RPM for 30 minutes. The supernatants were completely drained to leave the precipitates in the tubes that were then counted in a gamma counter and the plasma corticosterone levels were calculated.

2.6 Quantification and morphometric analysis

The motor neurons (n = 12 sections/bird) and their neurites in the lateral motor column (LMC) of the lumbosacral region of the spinal cord were counted examined using Axioplan II Imaging microscope (Carl Zeiss, New York) at 20X magnification. Quantification of motor neurons was performed for each segment of the lumbosacral region of the spinal cord using 40µm thick coronal sections for each sample. Images of the motor neurons were taken using a CCD camera (Orca-ER, Hamamatsu, Bridge water, NJ) connected to the microscope at 40X magnification. The morphometric analysis was performed using Image-Pro Plus 6.2. Cell Multipolar Shape Index -CMSI (cell roundness function – perimeter^2/4Πarea^2) of Image-Pro Plus was used to define the morphology of the motor neurons (n=10) between the groups (n=4/group). Motor neurons (n =40) in the lateral motor column were randomly selected for each bird. The number of neurites for cell body was counted. A mean count of neurites was then calculated which represented the typical number of neurites in motor neuron for each bird.

2.7 Statistical analysis

Statistical analyses were performed using software JMP® Pro 9.0 (SAS Institute Inc., NC). Differences among the groups were analyzed using one way analysis of variance (ANOVA). Pairwise comparisons between the groups were analyzed either using Student's t test or Tukey's (Honestly Significant Difference) HSD test. Data from each group are expressed as Mean ± SD.

3. Results

3.1 Experiment I - Macroscopic examination of the lower spinal cord and behavioral observations using a gait scoring method.

Samples of the synsacrum were dissected to expose the spinal cord embedded in bone. The synsacrum (Fig. 1a-c) is a bony structure formed by fusion of lumbar and sacral vertebrae and the bones of the pelvic girdle. The lower spinal cord, enclosed in the synsacrum, and the sciatic nerves from the spinal cord that innervates the leg muscles are shown in Fig. 1a-e. The synsacrum was dissected on the ventral side to expose the ventral view of the lower spinal cord and then to the dorsal side to expose the complete spinal cord (Fig 1e) with dorsal root ganglion attached to either side of each segment.

Using the five point gait scoring system the birds were gait scored and the prevalence of lameness rapidly advances after 4 weeks of age (Fig 2). Marked by the degree of lameness, gait score of '1' was found to be predominant at week 5 and gait scores of '1' and '2' were found to predominant at 6 and 7 weeks of age.

3.2 Experiment II –Examination of motor neurons in the lateral motor column of lumbosacral region 4 (LS 4) of the spinal cord in chicks from three different groups.

Histological examination using Nissl staining revealed that the morphology of the motor neurons in the lumbosacral spinal cord was largely globular in structure in the proposed neural

group showing lameness (Fig 3 a-d). This provided the first evidence that in lameness with normal leg bone condition, a neural dysfunction may be responsible for the reported 1.75% unknown cause of lameness in broilers. Interestingly, birds with lameness that had leg bone abnormalities, particularly FHS/FHN showed a typical star-shaped motor neurons and rare presence of globular-shaped motor neurons in the lower spinal cord. Presence of normal characteristics of motor neuron in the bone associated lame group distinctly separates them from the neural associated lame group. Immunostaining of the motor neurons with an antibody to ChAT likewise revealed a globular pattern of motor neurons and suggested a fewer number of neurites emerging from the perikarya in the neural associated lameness group (Fig 3f).

Quantification of the motor neurons in each segment of lumbosacral spinal cord (n=4) revealed that the number of motor neurons in the lumbosacral region 4 (LS 4) is highest than other segments of the lumbosacral region (Table 2). No significant difference was found among the three groups, $F_{2, 9} = 0.483$, $p = 0.631$, in the LS 4.

3.3 Experiment III – Quantification of motor neuron neurites in the lumbosacral region 4 (LS 4) of the spinal cord in chicks from three different lines of broilers, observation of body weight gain and assessment of stress level in three different groups.

A Significant decrease in the number of neurites in the neural associated lameness group was found in three different lines of broilers. In line B and C (n=85) the neural problem group (n = 4) showed a significant decrease in the number of neurites versus the control group, ($n = 4$; $p < 0.001$), and the bone associated lameness, ($n= 4$; $p = 0.010$), (Fig 4a). In line D (n=87) the neural associated lameness group (n=3) had decreased number of neurites versus the control group (n=4, $p = 0.021$) and bone associated lame group (n=3, $p = 0.017$) and was observed by pairwise comparison using Student's t test (Fig 4b). While in lines E and F (n=152) a significant decrease

in the number of neurites among the groups ($F_{2, 9} = 4.206$, $p = 0.051$) was found using ANOVA, however in this experiment both the bone (n=5, $p = 0.021$) and the neural associated lame (n=5, $p = 0.044$) group were significantly different from the control (n=3) group by pairwise comparison using Student's t test (Fig 4c).

Average percentage body weight gain over a 5 week period showed that the bone associated lameness group and neural associated lameness group had a lower body weight gain when compared to the control group (Fig 5a).

Corticosterone levels of the bone associated and neural associated lameness groups were increased threefold, $p < 0.05$, and fourfold, $p = 0.08$, respectively, when compared to the control group when observed by pairwise comparison using Student's t test (Fig 5b).

4. Discussion

The neural associated lameness with normal leg bones was observed in 3.7% of the total population of chickens used in this study. From the results of this study, a globular appearance of the motor neurons was observed along with a decrease in the number of neurites from the motor neurons in the lumbosacral spinal cord and the confirmation for the neural associated lameness was determined in each experiment after histological examination of the sections of the lumbosacral region. Interestingly, birds showing those abnormal characteristics, designated as neural associated lameness group, had significantly elevated corticosterone levels when compared to the controls.

The results from the trial experiments showed that age could be a major factor attributed to high prevalence of femoral head separation/necrosis, when the birds were sampled after 6-7 weeks of age. Studying broilers at an early age and also eliminating the femoral bone problem

helped to focus on lame birds with normal leg bones which could be caused by an abnormal neural condition (neural associated lameness). The absence of enlargement of the sciatic nerves and dorsal root ganglia in birds in the neural associated lameness group (supplementary material), suggest that an autoimmune disorder found in Leghorns causing peripheral neuropathy [21] was not observed in the neural associated lameness group or in any lame birds used in this study.

Prevalence of lameness increased after 4 week of age which was assessed using the behavioral scoring system, however the incidence of lameness varies between different lines of broilers, as it can be seen from the observations of the gait scores (supplementary material). Differences in susceptibility to lameness in different commercial lines of broilers have been noted in some studies [33-34].

Behavioral gait scoring systems were helpful in assessing the severity of lameness, however, the scoring systems were not helpful in differentiating birds into neural associated and bone associated lameness groups. In line D some neural associated lame birds were observed with a wide stance with a greater distance between the tibiotarsus of the legs as the birds walked through the runway. This characteristic gait could be a useful behavioral indicator for choosing the birds in the neural associated group if observed in further studies.

Morphological changes of motor neurons were observed in the neural associated lameness group, however, there was no evidence of a decrease in the number of motor neurons in either of the lame groups. The absence of differences among the groups in the number of motor neurons may be due to inconsistency in the selection of the birds with higher gait scores. To avoid interference with the bone associated lameness the birds were sampled as soon as they were observed with lameness using gait scoring system (GS>2). There is a possible combination of

neural and bone associated lameness which could have been eliminated due to high occurrence of bone problem at the time of sampling. As most of the birds were sampled at their earlier stages of the lameness, loss of motor neurons in the severe lame birds (GS =4) is still a question?

The decrease in the number of neurites in the motor neurons indicates that there could be a possible degeneration of neurites of the motor neurons affecting the connections between the motor neurons which would affect the normal gait. Differences in the number of neurites in the bone and the neural associated lameness groups when compared to the controls in the mixed lines (E and F) raises a question of whether there is an association between the neural and the bone group. It has been reported that stress can affect the intracellular signaling mechanism in the sensory neurons [35] and some studies have described that insults to the sensory system have significant effect on the bone metabolism [36-37]. It is thus possible that the nervous system can affect bone metabolism by stress. Corticosterone level was increased fourfold in the neural associated lame group which indicates a high level of stress in this group of birds. It can be seen that neural problem birds have decreased number of neurites. It has been reported that chronic stress can affect neurites as well as neurons in the hippocampus [38] and modulates motor functions in the vertebrates [39]. Thus, it is possible that the decrease in the number of neuritis and the impairment in the locomotion of broilers could be influenced by stress. Studies have shown an association between the decreased body weight gain and increased corticosterone level in broilers and laying hens [40-42]. The lower percentage body weight gain and the increased blood plasma corticosterone level of the lame birds suggest that a bird in pain would not be able to move and have food and water.

Studies in human leg disorders have shown that motor neurons can be affected to different degrees, from reduction in the length of the neurites of motor neurons [43] to degeneration of

motor neurons [44]. For example, in spinal muscular atrophy (SMA), denervation of motor neuron axons to the muscles causes leg weakness [45] and in amyotrophic lateral sclerosis (ALS), degeneration of motor neurons in the anterior horn of the lumbar region of the spinal cord leads to walking difficulties [44,46]. If these types of degenerative pathology are observed in broilers there are possibilities of using chickens as biomedical models for degenerative disorders in humans.

In conclusion, it has been found that the change in the morphology of the motor neurons and the decreased number of neurites in the motor neurons of the lumbosacral spinal cord is associated with lameness in broilers having normal leg bones.

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Table 1. Five point gait scoring system used for assessing the severity of lameness in broilers that were raised on wire floor pens.

Gait Score (GS)	Description
0	Normal gait, takes several steps before stopping in an open field or a runway.
1	Uneven gait, favors one leg or shows side to side wobble.
2	Uneven gait coupled with sitting (one leg favored or wobble is more obvious). Able to take several steps if prodded repeatedly.
3	Takes a step or two with imbalance and squats.
4	Cannot stand nor walk.

Table 2. Average number of motor neurons in each segments (LS1-LS8) of the lumbosacral spinal cord of chickens (n=4)

Lumbosacral segment (LS)	Right side cell count [a]	Left side cell count [a]
LS 1	21.96 ± 0.82	22.43 ± 0.86
LS 2	33.96 ± 0.73	32.93 ± 0.78
LS 3	38.97 ± 0.70	39.00 ± 0.68
LS 4	45.19 ± 0.64	44.33 ± 0.64
LS 5	40.41 ± 0.89	39.23 ± 0.84
LS 6	28.88 ± 0.98	29.48 ± 0.78
LS 7	15.76 ± 0.71	16.15 ± 0.76
LS 8	10.07 ± 0.50	9.34 ± 0.58

a - Mean ± SE (n=4).

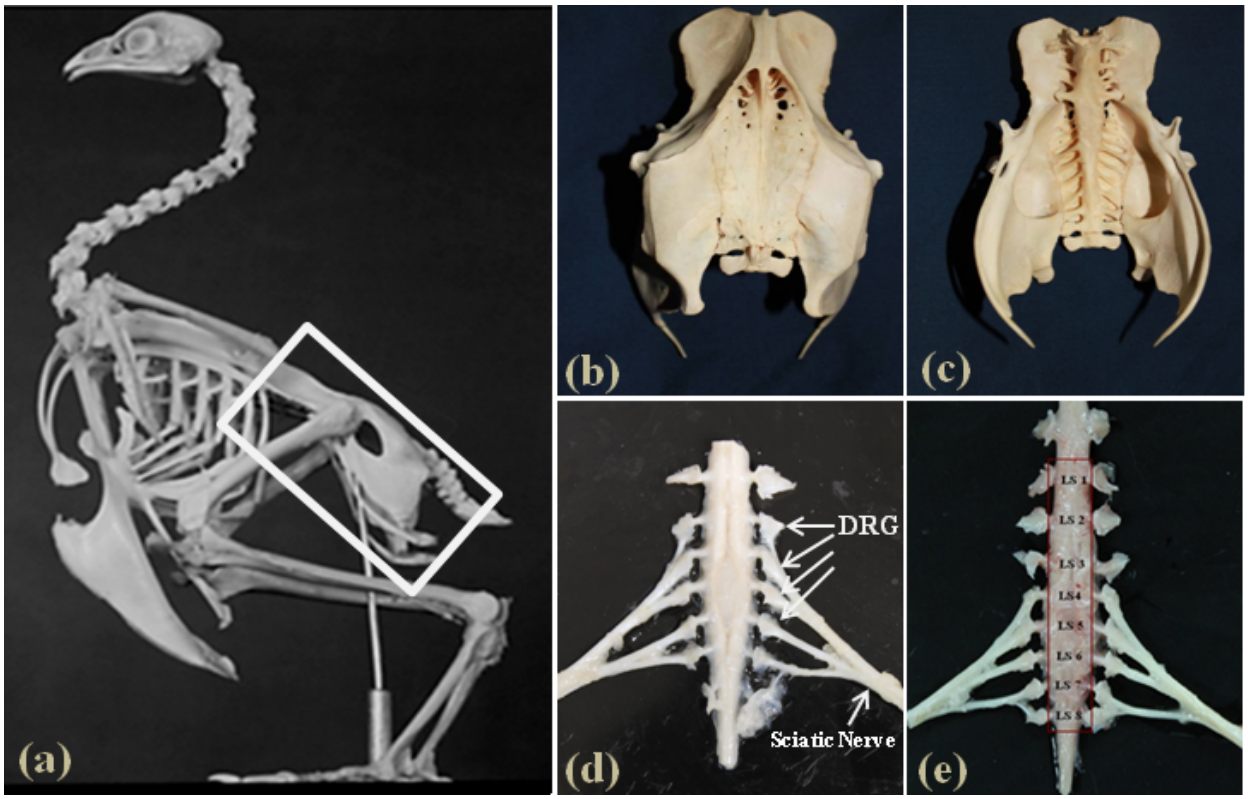

Fig. 1a-e. Synsacral region and the lower spinal cord of chickens. (1a) Lateral view of the skeleton of chicken. The boxed area shows the synsacral region which encloses the lower spinal cord. (1b) Doral view of the synsacrum. (1c) Ventral view of the synsacrum. (1d) Dissected sacral region (used in trial experiments) of the spinal cord from synsacral region showing the dorsal root ganglion (DRG) and sciatic nerve formed from five sacral segments of the spinal cord (1e) Fully dissected lumbosacral region of the spinal cord designated by the nomenclature LS1-LS8.

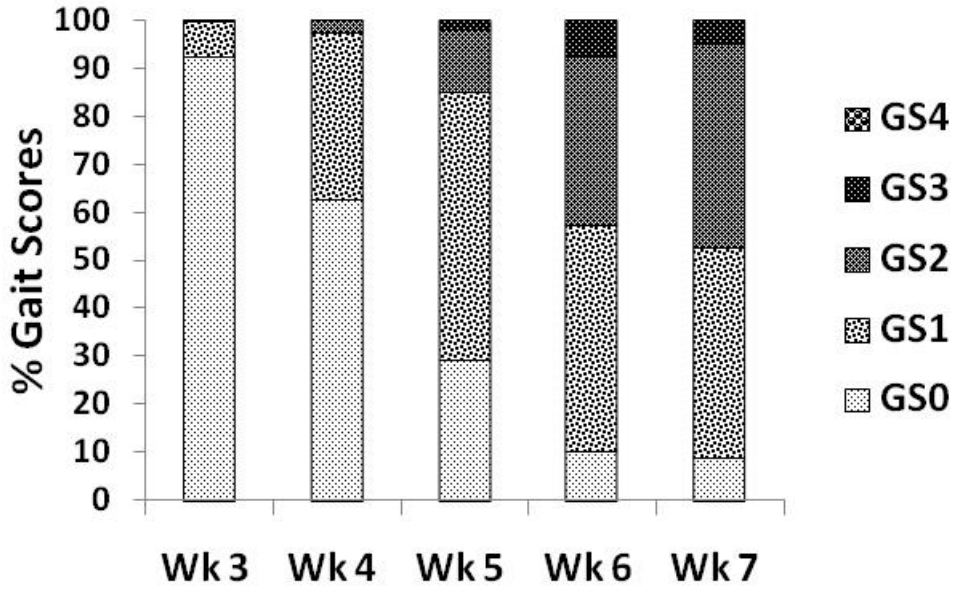

Fig. 2. Percentage of gait scores based on weekly behavioral observations: Behavioral observations using the five point gait scoring system with gait score (GS) 0 being normal to gait score (GS) 4 being severely lame. Lameness in the broiler chickens from mixed lines E and F (n = 152) observed from week 3 to week 7 advances more rapidly after 4 weeks of age.

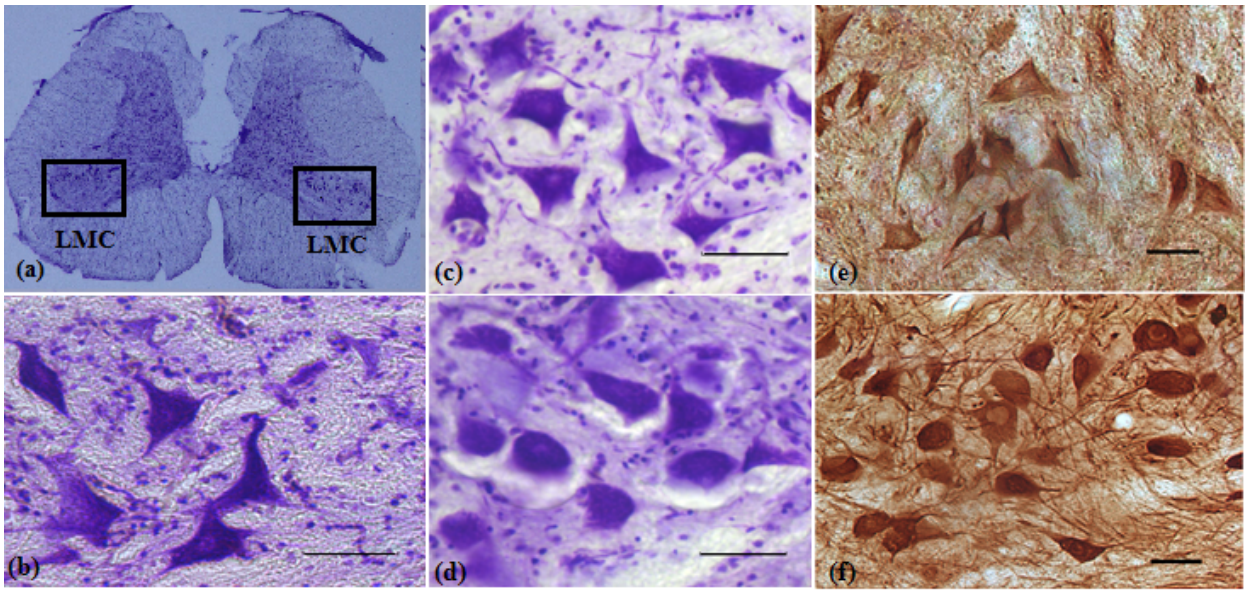

Fig. 3. Lumbo sacral segment 4 (LS4) of the spinal cord: (a) the lateral motor column (LMC) is shown in the box in a cross section of a spinal cord, (b) and (c) show normal motor neurons in the LMC bone associated lame group and control group, respectively, while (d) shows the globular neurons in the proposed neural associated lameness group (Nissl staining). (e) and (f) shows motor neurons in the LMC, immunostained with Choline Acetyl Transferase antibody, from a control bird and a proposed neural associated lame bird – showing globular neurons, respectively. Scale bar - 50µm.

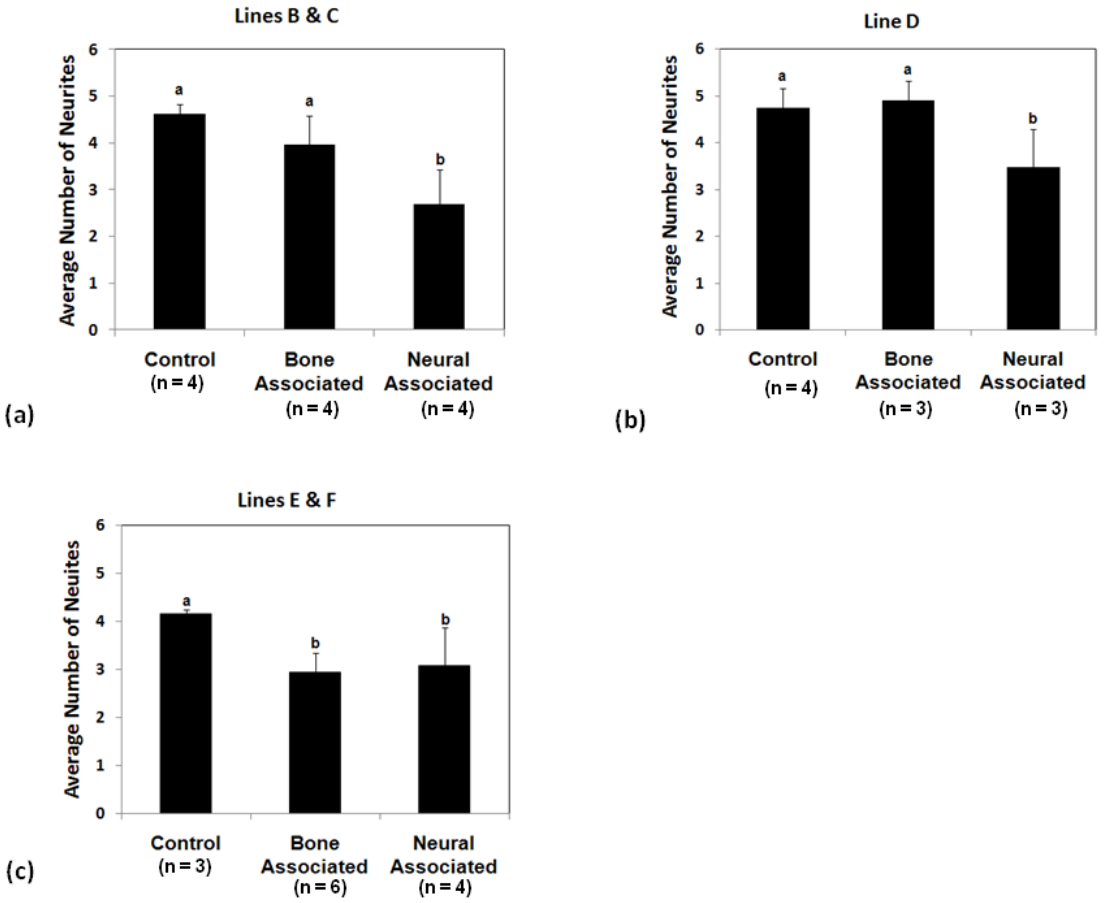

Fig. 4. Average number of neurites or processes from the cell body of motor neurons in three groups – Control, Bone Associated Lameness (lame bird with leg bone pathology) and Neural Associated Lameness (lame bird with normal leg bones) from different lines of broiler chickens. Error bar – SD. Significance level $p < 0.05$. Same letter within each bar graph shows group means are not significantly different ($p > 0.05$).

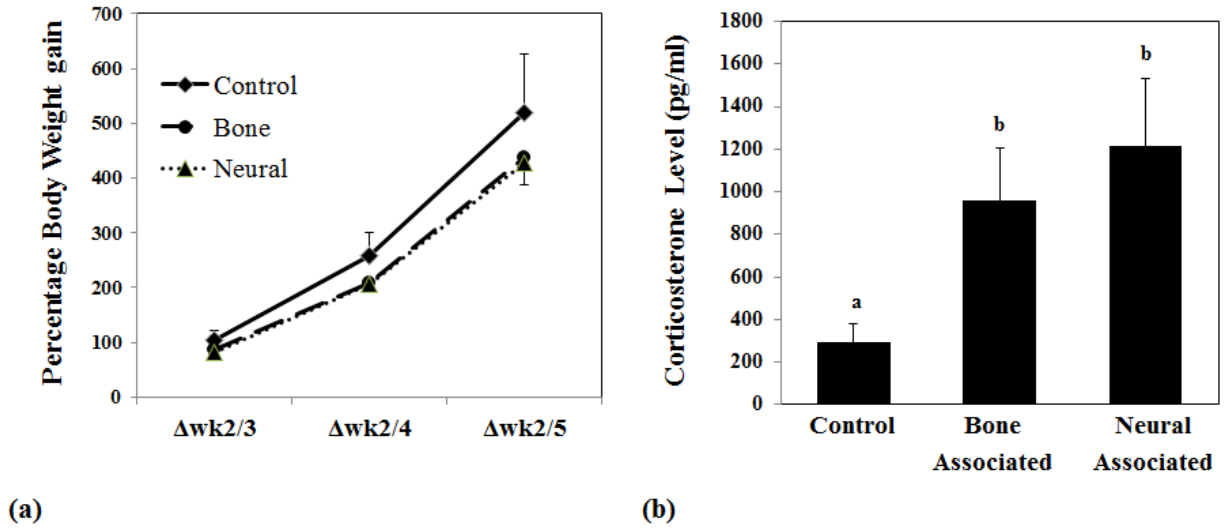

Fig. 5. Association between body weight gain and stress level (a) Percentage of body weight gain was measured for week 3, 4 and 5 using the base body weight measurement from week 2 (ΔWk2/3, ΔWk2/4 and ΔWk2/5). (b) Blood plasma corticosterone concentration was higher in lame birds when compared to controls (n=4). The decreased body weight gain and increased corticosterone level indicate an inverse relationship between stress and growth rate from week 3-5. Error bar – SD. Same letter within the bar graph shows group means not significantly different ($p > 0.05$).

6. Supplementary material

Trial I: M*acroscopic observations after necropsy:* Using the commercial gait scoring system birds were differentiated into controls (n=6) and lame (n=6) at 8 weeks of age. Femoral head separation/necrosis (n=11) was found in all the birds except one control bird.

Trial II: Neuroanatomical examination of lameness with normal bone condition and histological examinations of coronal sections of spinal cord: Using the commercial scoring system, a total of 8 lame birds was sampled at 5 weeks of age. Out of 8 sampled lame birds only one bird was affected with femoral separation/necrosis and the remaining 7 birds had normal femur head (Fig.1) and tibial head. The lame birds with normal bone after necropsy were defined as unidentified lame group. The broilers used for this experiment were not observed with inflammatory signs in dorsal root ganglions and sciatic nerves (Fig. 2) and also histological examination revealed that infiltrations of white blood cells were not found in the sections of spinal cord and dorsal root ganglion of the lame birds. To repeat the observation of lame birds with normal bone condition at 5 weeks of age, another set of birds were sampled from the same at 8 weeks of age, control (n=15) and lame birds (n=14). Femoral head separation/necrosis was found in 25 birds, including controls (n=11) and all the lame birds (n=14).

Hence, from trial I and II it was observed that after 6-7 weeks of age there is an increased prevalence of lameness with femoral head necrosis/separation.

Table 1. Commercial Gait scoring system used to differentiate normal vs. lame birds, adapted from Webster et al., 2008.

Gait Score (GS)	Description
0	Birds have balanced gait and can walk at least 10 steps
1	Able to walk at least 10 steps but with limping or awkward gait
2	Not able to make 10 steps and may use its wings and shank for mobility

Table 2. Gait scores adapted from Kestin's gait scoring system for assessing severity or different degrees of lameness (Kestin et al., 1992).

Gait Score (GS)	Description
0	Smooth and no Identifiable disability in gait
1	Slight defect that is difficult to observe such as uneven gait
2	Clear visible defect in gait which does not affect the birds mobility for foraging
3	Identifiable defect in gait, such as limping, jerky or unsteady stride, splaying of leg, which affects the birds mobility and prefer to squat if not forced to move
4	Severe identifiable defect in gait and bird squats within few steps if not strongly motivated and the mobility is severely affected
5	Inability to walk but the mobility can be achieved with the help of wing or with slow movement using shanks

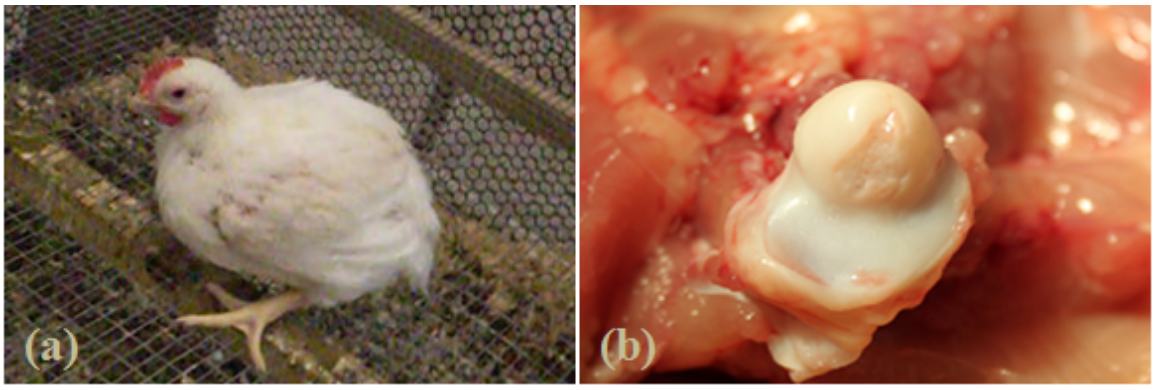

Fig. 1. Lameness in broiler chickens was observed with normal leg bones (a) lame bird sitting on its hocks and (b) lame bird had normal femoral bone when the prevalence of lameness observed with the femoral head separation/necrosis was high.

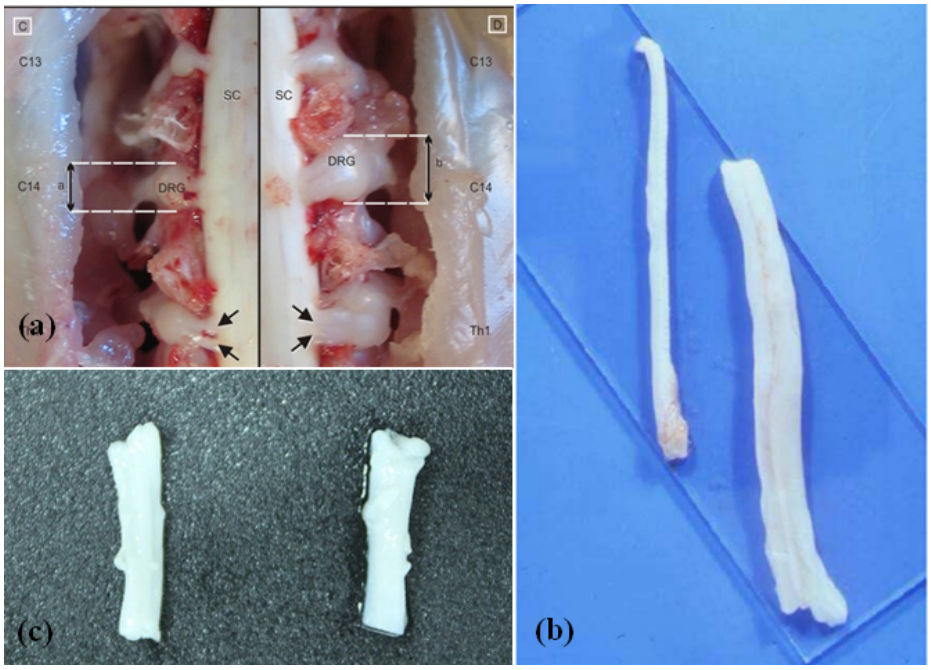

Fig. 2. Clock wise (a) Inflammation of the dorsal root ganglion (DRG) and spinal roots in CIDP (chronic Inflammatory Demyelinating Polyneuropathy) can be observed in the right when compared to the control in the left (from Bader et al., 2010) and (b) Enlargement of the peripheral nerve an inflammatory sign in polyneuropathy in Leghorns can be seen in the sciatic nerve (from Philipp et al., 2008 – unpublished) (c) Sciatic nerve samples collected near the proximal part of femur. Enlargement of the sciatic nerve was not observed in the unidentified group (right) that could not walk compared to control (left).

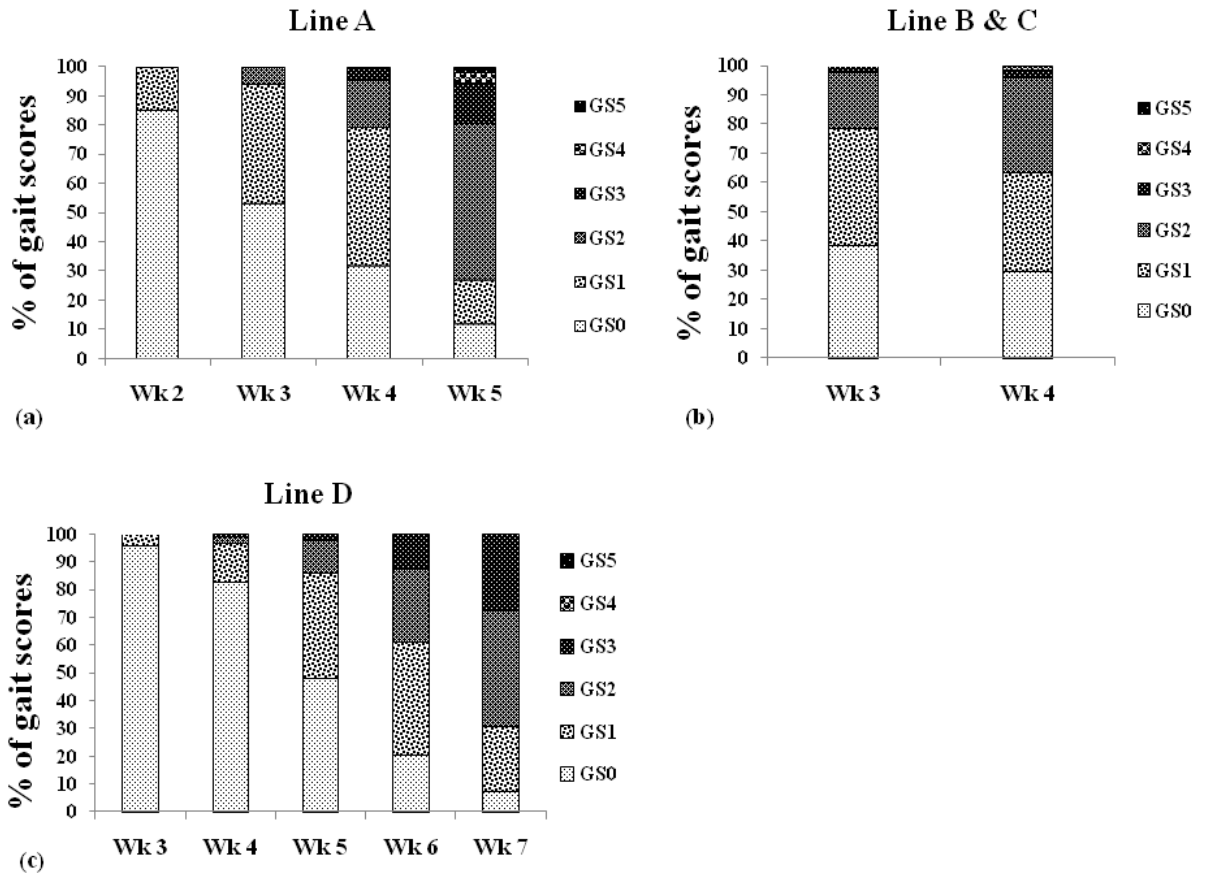

Fig. 3. Percentage of gait score based on weekly behavioral observations using Kestin's six point gait scoring system: Differences in the prevalence of lameness can be observed in three different lines of broiler chickens (a) Line A, n = 68, (b)Mixed line B and C, n= 85 and (c) line D, n=87. Some lines of broiler chickens shows progression as early as 3 weeks and some shows after 5 weeks of age, indicating that some lines are susceptible and some are resistant to lameness.

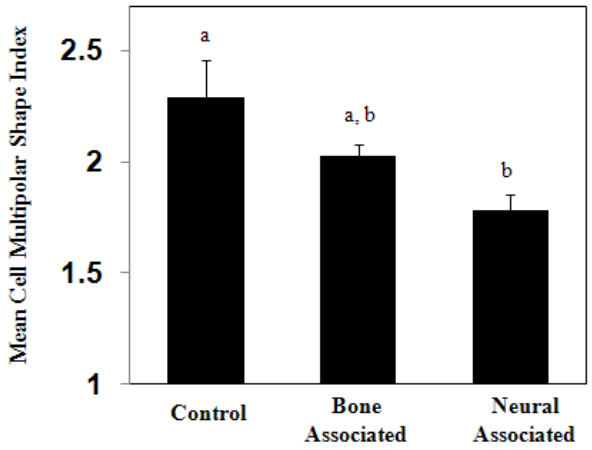

Fig. 4. Cell Multipolar Shape Index (CMSI) function – CSMI was measured by Image Pro-plus 6.0 (perimeter^2/4Πarea). Values closer to 1 indicate cells are globular or rounded in structure. Significant difference among the groups was found using ANOVA in the cell multipolar shape index, $F_{2, 9} = 5.668$, $p = 0.024$. The neural associated lameness group is significantly different from the control group in the cell multipolar shape index, $p = 0.019$ by pairwise comparison using Tukey's HSD test. The error bars in the graph indicates SE.

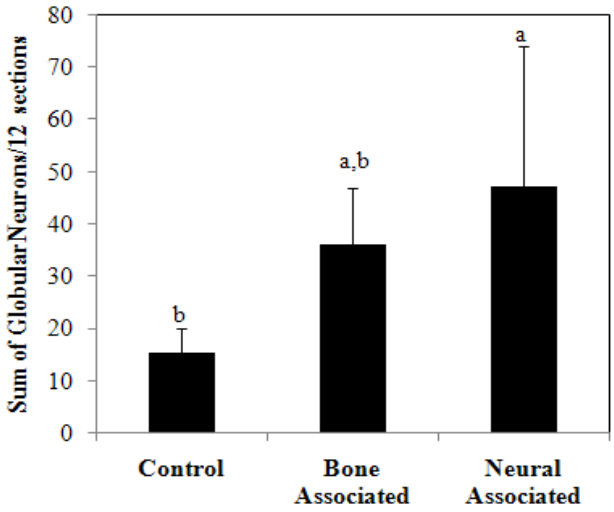

Fig. 5. Differences in the number of globular pattern neurons in the lumbosacral segment 4 of the spinal cord. The number of globular neurons in the neural associated lame group (n=5, $p = 0.041$) was significantly higher when compared to the control group (n=3), by pairwise comparison using Student's t test. The error bars in the graph indicates SD.

CONCLUSION

Behavioral gait observations and necroscopic examination revealed that there are differences in the prevalence and cause of lameness in different lines of broilers. Elevated blood plasma corticosterone levels in lame birds indicate the severity of pain associated with lameness in broilers irrespective of its type. From the results of this study it has been found that the morphology of the motor neurons in the lumbosacral spinal cord in the neural associated lame birds (with normal leg bones) were globular in structure when compared to the normal multipolar star shaped structure of motor neurons in the control birds and the bone associated lame birds. The results also revealed that the numbers of neurites or processes of the motor neurons have been reduced significantly in the lumbosacral spinal cord of the neur al associated lame birds with normal leg bones. Thus the data presented herein suggest that a neural dysfunction is responsible for certain types of lameness in broilers and it is independent of bone associated lameness. Further studies need to be accomplished to understand the actual cause of the neural associated lameness in young broilers.