ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

International Journal of Poultry Science 11 (7): 427-432, 2012
ISSN 1682-8356

Histological Changes in the Broiler Embryonic Pipping Muscle Between Days 15 and 19 of Incubation[1,2]

R. Pulikanti[1], E.D. Peebles[1], A.O. Sokale[1] and W.R. Maslin[2]
[1]Department of Poultry Science, [2]College of Veterinary Medicine,
Mississippi State University, Mississippi State, Mississippi, 39762, USA

Abstract: Nutritional and metabolic changes in the avian pipping muscle have been discussed by previous researchers. However, there are no reports in the literature on the histology of the embryonic pipping muscle in modern broiler strains. Therefore, the current experiment was conducted to examine histological changes in the embryonic pipping muscle of a modern broiler strain between d 15 and 19 of incubation. Ross x Ross 708 broiler hatching eggs were incubated on 8 replicate tray levels of an incubator. On d 15 and 19 of incubation, 2 embryos per level were extracted and their head and neck portions were preserved. The tissues were processed and stained using standard histological techniques. Subsequently, longitudinal and transverse sections of the embryonic pipping muscles on each of those days were examined under 2x, 4x, 10x, 20x and 40x magnifications. In preparation for hatch between d 15 and 19 of incubation, muscle fiber thickness increased, suggesting protein accretion and nutrient accumulation in the individual muscle fibers. Intra-fascicular muscle fiber density decreased and the inter-fascicular spaces widened and were filled with more cellular and fluid components, suggesting the active and selective infiltration of lymph into the pipping muscle from the surrounding lymph glands. In addition, the inter-fascicular spaces were filled with more cellular debris, which may be a result of muscle cell degeneration, necrosis, or associated apoptotic changes in the actively growing pipping muscle. Results of the current experiment provide an insight into the morphological changes in the pipping muscle during embryogenesis in a modern broiler strain. These together with the other associated changes in the nutritional profiles and the proteome compositions of the pipping muscle, as previously reported from our laboratory, facilitate a more detailed and comprehensive understanding of the various orchestrated cellular, metabolic and physiological events that occur in the pipping muscle of a modern broiler strain during the later part of incubation as the embryo prepares for hatch.

Key words: Broiler, embryo, histology, lymph, pipping muscle

INTRODUCTION

Emergence of the broiler embryo as a hatchling involves the use of a specialized muscle (muscularis complexus) that is located on the antero-dorsal cervical region of its neck, which is also commonly referred to as the pipping or hatching muscle (Romanoff, 1960; Bock and Hikida, 1968; Parkhurst and Mountney, 1988). Various other muscles located on the neck of the embryo, namely, the musculus biventris, musculus spinalis and splenius cervicis also contribute to the embryo's efforts to hatch (Bock and Hikida, 1968). The pipping muscle originates on the cervical vertebrae and inserts onto the posterior portion of the parietal bones (Smail, 1964). The pipping muscle is bounded on either side by lymph glands. As the embryo prepares for hatch, the pipping muscle enlarges in size by the selective infiltration of lymph from the surrounding lymph glands. The muscularis complexus reaches its maximum size by d 19 or 20 of incubation (Pohlman, 1919) and gains maximum turgidity due to the selective infiltration of lymph (Fisher, 1958). Subsequent to hatch, the pipping muscle looses excess lymph and regresses to its original size within a few d post-hatch (Fisher, 1958).

Although previous researchers have examined the gross, microscopic and ultra-microscopic structures of the pipping muscle, many of these studies were conducted approximately 35 yr ago and therefore, did not include modern broiler strains. Furthermore, those studies were conducted using domesticated chickens such as the Rhode Island Red (Fisher, 1958), White Leghorn (Rigdon *et al*., 1968; Bock and Hikida, 1968), White Plymouth Rock (Hayes and Hikida, 1976) and New Hampshire (Rigdon *et al*., 1968) breeds, which are in contrast to the highly selected broiler strains available today. Modern commercial broiler strains are considered genetically superior to the chickens used in these earlier studies, because they are able to reach market age faster and produce higher processing meat yield. Consequently, embryos of the modern broiler strains may also grow relatively faster over a given length of

Corresponding Author: E.D. Peebles, Department of Poultry Science, Mississippi State University, Mississippi State, Mississippi, 39762

incubation. Pulikanti *et al.* (2010) further suggested that the pipping muscle of embryos in modern broiler strains exhibit a more rapid rate of growth through d 19 of incubation in comparison to those reported by Pohlman (1919) and Fisher (1958).

As a functional pipping muscle is critical for the hatching process (Romanoff, 1960; Smail, 1964), it would be advantageous to learn the various morphological and physiological events that occur in the pipping muscle of the modern broiler strain embryo as it prepares to hatch. Although the growth pattern of the pipping muscle during embryogenesis has been assessed through weighing and gross visual observation by previous researchers (Pulikanti *et al.*, 2010, 2011a,b), these researchers did not further investigate the specific histological changes that occur in the pipping muscle of modern broiler strains during the later phase of incubation. Therefore, the objective of the current experiment was to examine the histological changes in the pipping muscle of modern broiler strain embryos between d 15 and 19 of incubation. Pipping muscles on d 15 of incubation were chosen because d 15 is the earliest time when pipping muscle size is adequate and when it is distinguishable from its underlying tissues. Also, pipping muscles on d 19 were chosen because d 19 is when the embryo initiates the hatching process and when pipping muscle turgidity and metabolic activity are maximal (Pulikanti *et al.*, 2010). Furthermore, the information obtained from this study will be used in conjunction with the results of other companion studies conducted in our laboratory that examined changes in the nutritional profiles (Pulikanti *et al.*, 2010) and proteome compositions (Sokale *et al.*, 2011) of the pipping muscles during the later period of embryogenesis.

MATERIALS AND METHODS

Four hundred and eighty Ross x Ross 708 broiler hatching eggs were obtained from a 30-wk-old breeder flock and were incubated on 8 replicate tray levels (60 eggs per level) of a Jamesway Model 500 single stage incubator (Jamesway Incubator Company, Inc., Cambridge, ON, Canada), under standard commercial conditions at 37.5°C dry bulb and 28.8°C wet bulb temperatures. On d 15 and 19 of incubation, the eggs were broken out to extract 2 live embryos per level. The embryos were then surgically dissected and the head and neck portions were carefully excised without disrupting the morphological features of the associated structures. The tissues were immediately preserved in 10% neutral buffered formalin to prevent autolysis and microbial decomposition. The tissues were fixed in 10% neutral buffered formalin for at least 72 h. Subsequently, the head and neck portions of the embryos were placed in Kristensen's decalcifying solution (1:1 mixture of 8 N formic acid and 1 N sodium formate; Kristensen, 1948) to remove calcium from bones and other calcified tissues. The decalcification was continued until the tissues were determined to be free of calcium. Two sections were prepared from each embryo. The first preparation was a paramedian sagittal section of the entire head and proximal cervical region. The rostral end of the head was then trimmed back so that it would fit in a standard processing cassette. Subsequently, a second preparation was a transverse section which was cut through the center of the pipping muscle from the larger remnant half of the embryo. Both preparations were approximately 3 to 4 mm in thickness. Following this, the cassettes containing the tissue sections were washed in gently running water for 24 h to remove residual acid and then placed in 10% neutral buffered formalin until processed. All tissues were processed routinely, embedded in paraffin, sectioned at 6 μm and stained with hematoxylin and eosin (Harris, 1900; Mallory, 1938). The slides containing the longitudinal and transverse sections of the d 15 and 19 embryonic pipping muscles were examined using a compound microscope set at 2x, 4x, 10x, 20x and 40x magnifications, respectively. The observed fields were photographed and were examined for histological changes that occurred in the pipping muscles between d 15 and 19 of incubation.

RESULTS AND DISCUSSION

The histological structures of the pipping muscles in 15 and 19 d broiler embryos are presented in Fig. 1 and 2, respectively. Figures 1A through 1E represent the longitudinal sections of the pipping muscle in a 15-d-old embryo, under 2x, 4x, 10x, 20x and 40x magnifications, respectively; whereas, Figures 1F through 1J represent the longitudinal sections of the pipping muscle in a 19-d-old embryo, at 2x, 4x, 10x, 20x and 40x magnifications, respectively. Based on the histological structures as observed under the 2x, 4x and 10x magnifications, one can visually appreciate differences in the d 15 pipping muscle when compared to that of the d 19 pipping muscle. More specifically, the d 19 pipping muscle contained thicker muscle fibers, whereas, their intra-fascicular muscle fiber densities were lower compared to the d 15 pipping muscle. Further examination of the histological structures under 20x and 40x magnifications revealed that compared to the d 15 pipping muscle, the inter-fascicular spaces of the day 19 pipping muscle were wider and were filled with more cellular debris and infiltrated cells.

The transverse sections of the pipping muscle in a 15-d-old embryo under 2x, 4x, 10x, 20x and 40x magnifications are presented in Fig. 2K through 2O, respectively; whereas the transverse sections of the pipping muscle in a 19-d-old embryo under 2x, 4x, 10x, 20x and 40x magnifications are presented in Fig. 2P through 2T, respectively. Although the exact sizes of the individual muscle fibers or muscle fiber bundles were not

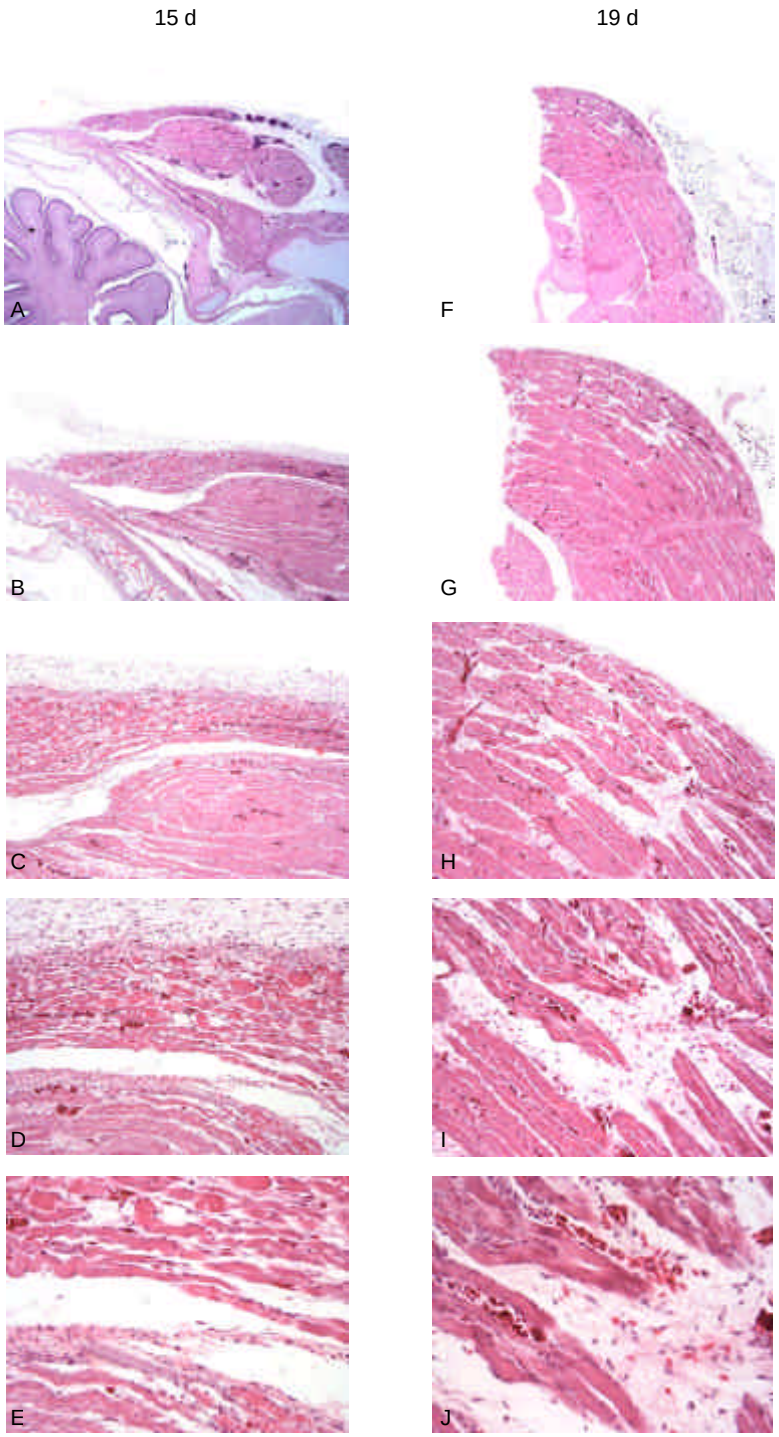

Fig. 1: Histological structure of broiler embryonic pipping muscle in a longitudinal view on *15 and †19 d of incubation at 2x (*A, †F), 4x (*B, †G), 10x (*C, †H), 20x (*D, †I) and 40x (*E, †J) magnifications, respectively

determined in this experiment, one can visually appreciate the histological changes that occur in the pipping muscle between 15 and 19 d of incubation in these transverse sections. The observations in the transverse sections were similar to those in the longitudinal sections under the different magnifications. Compared to the d 15 pipping muscle, the d 19 pipping muscle contained thicker muscle fibers that were less

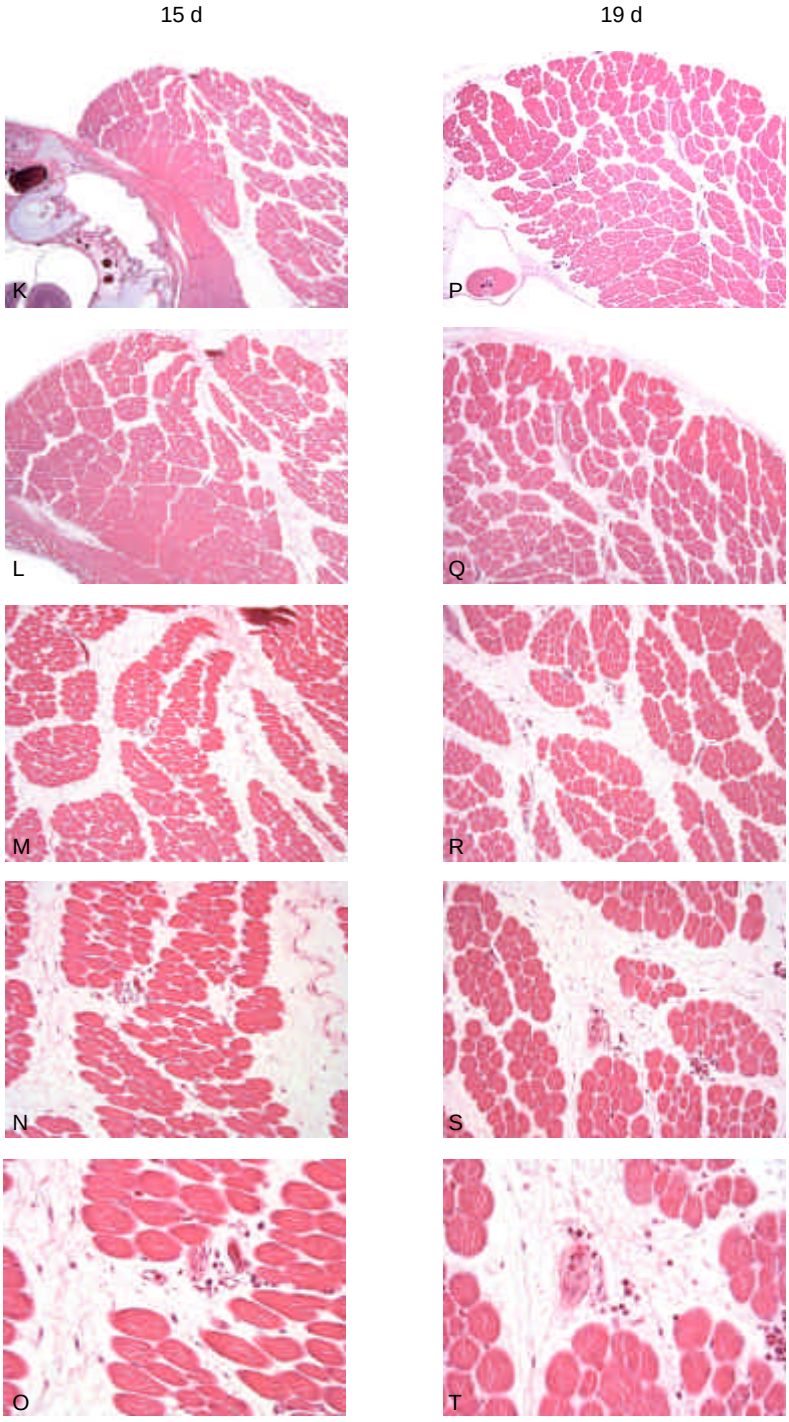

Fig. 2: Histological structure of broiler embryonic pipping muscle in a transverse view on *15 and †19 d of incubation at 2x (*K, †P), 4x (*L, †Q), 10x (*M, †R), 20x (*N, †S) and 40x (*O, †T) magnifications, respectively

densely distributed in the individual muscle fiber bundles. Further observations of the transverse sections under 20x and 40x magnifications revealed that compared to the d 15 pipping muscle, the spaces between the muscle fiber bundles in the d 19 pipping muscle were wider, contained more cellular debris and had greater numbers of infiltrated cells.

An increased thickness of the individual muscle fibers indicates active muscle tissue accretion in the pipping muscle during the later part of incubation, as suggested

by Fisher (1958). In addition, increased size of the inter-fascicular spaces and increases in the cellular and fluid components in those spaces indicate the active and selective infiltration of lymph into the pipping muscle from the surrounding lymph glands during the later part of incubation, as suggested by Pohlman (1919). Furthermore, increased cellular debris in the inter-fascicular spaces of the pipping muscle may have resulted from muscle cell degeneration (Bock and Hikida, 1968; 1969; Hayes and Hikida, 1976; Allen, 1984), necrosis (Rigdon *et al*., 1968), or associated apoptotic changes (McClearn *et al*., 1995). Upon visual comparison of the pipping muscle transverse sections in the current report with those at a similar magnification in meat-type chicks by Fisher (1958) on d 16 of incubation, it was noted that inter-fascicular and intra-fascicular spaces were larger in those from the current study; whereas on d 19, the morphologies of the muscle appeared similar, suggesting that an earlier onset of lymph infiltration may have occurred in the Ross x Ross 708 pipping muscle.

However, the pattern of changes in the pipping muscles between the designated time periods are essentially the same as those shown by Fisher (1958). It is, therefore, suggested that despite an earlier onset of lymph infiltration in response to genetic selection for a higher growth rate in modern broilers, the overall pattern of morphological changes in the pipping muscle remained largely unaffected.

The increased thickness of individual muscle fibers between 15 and 19 d of incubation, as observed in this study, may be a result of protein accretion in the pipping muscle by the active utilization of the yolk sac nutrients (Pulikanti *et al*., 2010). Moreover, Pulikanti *et al*. (2010) also suggested that between 15 and 19 d of incubation, the pipping muscle accumulates energy reserves in the form of glucose and glycogen as the broiler embryo prepares for hatch. An examination of the exact distribution of these individual nutrients into different cellular compartments may provide further in-depth information regarding the various orchestrated events that occur in the pipping muscle as the embryo prepares for hatch.

Nevertheless, the observed histological changes in the pipping muscles under different magnifications, as described in this manuscript, provide useful information regarding morphological changes that occur in the pipping muscle of modern broiler strains during the later part of embryogenesis. These together with the results reported from other companion experiments in our laboratory, which investigated changes in the nutritional profile (Pulikanti *et al*., 2010) and proteome composition (Sokale *et al*., 2011) of the pipping muscle, may facilitate a more comprehensive understanding of the various orchestrated cellular, metabolic and physiological events that occur in the pipping muscle as the broiler embryo prepares for hatch.

ACKNOWLEDGEMENTS

We express our appreciation for the expert technical assistance of Sharon K. Womack of the Mississippi State University Poultry Science Department.

REFERENCES

Allen, E.R., 1984. The musculus complexus of normal and dystrophic chicken embryos. Poult. Sci., 63: 2087-2093.

Bock, W.J. and R.S. Hikida, 1969. Turgidity and function of the hatching muscle. Am. Mid. Nat., 81: 99-106.

Bock, W.J. and R.S. Hikida, 1968. An analysis of twitch and tonus fibers in the hatching muscle. The Condor, 70: 211-222.

Fisher, H.I., 1958. The hatching muscle in the chick. The Auk, 75: 391-399.

Hayes, V.E. and R.S. Hikida, 1976. Naturally-occurring degeneration in chick muscle development: Ultrastructure of the M. complexus. J. Anat., 122: 67-76.

Harris, H.R., 1900. On the rapid conversion of haematoxylin into haematin in staining reactions. J. Appl. Microsc., 3: 777-780.

Kristensen, H., 1948. An improved method of decalcification. Stain Technol., 23: 151-154.

Mallory, F.B., 1938. Pathological techniques. Philadelphia: W.B. Sauders Company.

McClearn, D., R. Medville and D. Noden, 1995. Muscle cell death during the development of head and neck muscles in the chick embryo. Dev. Dynam., 202: 365-377.

Pohlman, A.G., 1919. Concerning the causal factor in the hatching of the chick, with particular reference to the musculus complexus. Anat. Rec., 17: 89-104.

Pulikanti, R., E.D. Peebles, R.W. Keirs, L.W. Bennett, M.M. Keralapurath and P.D. Gerard, 2010. Pipping muscle and liver metabolic profile changes and relationships in broiler embryos on days 15 and 19 of incubation. Poult. Sci., 89: 860-865.

Pulikanti, R., E.D. Peebles and P.D. Gerard, 2011a. Physiological responses of broiler embryos to *in ovo* implantation of temperature transponders. Poult. Sci., 90: 308-313.

Pulikanti, R., E.D. Peebles and P.D. Gerard, 2011b. Use of implantable temperature transponders for the determination of air cell temperature, eggshell water vapor conductance and their functional relationships in embryonated broiler hatching eggs. Poult. Sci., 90: 1191-1196.

Parkhurst, C.R. and G.J. Mountney, 1988. Poultry meat and egg production. Van Nostrand Reinhold Company, New York.

Rigdon, R.H., T.M. Ferguson, J.L. Trammel, J.R. Couch and H.L. German, 1968. Necrosis in the pipping muscle of the chick. Poult. Sci., 47: 873-877.

Romanoff, A.L., 1960. The avian embryo. The Macmillan Co., New York.

Sokale, A., E.D. Peebles, W. Zhai, T. Pechan and S. Burgess, 2011. Proteome profile of the pipping muscle in broiler embryos. Proteomics, 11: 4262-4265.

Smail, J.R., 1964. A possible role of the musculus complexus in pipping the chicken egg. Am. Mid. Nat., 72: 499-506.

[1]This is Journal Article Number J-12175 from the Mississippi Agricultural and Forestry Experiment Station supported by MIS-322210.

[2]Use of trade names in this publication does not imply endorsement by Mississippi Agricultural and Forestry Experiment Station of these products, nor similar ones not mentioned.