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
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% 
normal 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-
fascular 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
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.
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.
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.
Kristensen, H., 1948. An improved method of
Philadelphia: W.B. Saunders Company.
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,
muscle and liver metabolic proﬁle changes and
relationships in broiler embryos on days 15 and 19
Physiological responses of broiler embryos to in
ovo implantation of temperature transponders.
of implantable temperature transponders for the
determination of air cell temperature, eggshell
water vapor conductance and their functional
relationships in embryoated broiler hatching eggs.


